SUPPLEMENTAL DATA

Chemical synthesis

General experimental methods

All reagents and solvents were used as received from their commercial source without further purification. Regarding the ascorbic acid solution, a pharmaceutical solution of 25% aqueous ascorbic acid approved by the Japanese Pharmacopoeia was used. [^{11}C]Carbon dioxide was produced by a ^{14}N(p,α)^{11}C nuclear reaction using a 12-MeV cyclotron (CYPRIS HM-12S Cyclotron, Sumitomo Heavy Industry). [^{11}C]CH_3I was prepared by reduction of [^{11}C]carbon dioxide with lithium aluminum hydride followed by iodination with hydroiodic acid using proprietary automated radiolabeling at RIKEN, which consisted of heating the reaction mixture, dilution, HPLC injection, fractional collection, evaporation, and sterile filtration. Radioactivity was quantified with a dose calibrator. Semi-preparative purification and purity analysis by HPLC was performed on a system equipped with pumps and a UV detector, and radioactivity in the effluent was determined using a radio analyzer.

Synthesis of (*RS*)-[^{11}C]KTP-Me

According to the previous report (9), (*RS*)-[^{11}C]KTP-Me was synthesized by using

approximately 20 GBq of [^{11}C]CH_3I. The total synthesis time, including HPLC purification and radiopharmaceutical formulation, was less than 25 min. The [^{11}C]CH_3I-based decay-corrected radiochemical yield was calculated to be 29–47%. The chemical identity of (*RS*)-[^{11}C]KTP-Me was confirmed by co-injection with an authentic sample of ketoprofen methyl ester using analytical HPLC (mobile phase: acetonitrile/water = 50:50; column: COSMOSIL, 5C_{18}-AR-II, 4.6 mm internal diameter (i.d.) × 100 mm, 5 μm; flow rate: 1.0 mL/min; UV detection: 255 nm; retention time: 7.8 min).

Separation of (*R*)-[^{11}C]KTP-Me with the chiral HPLC method

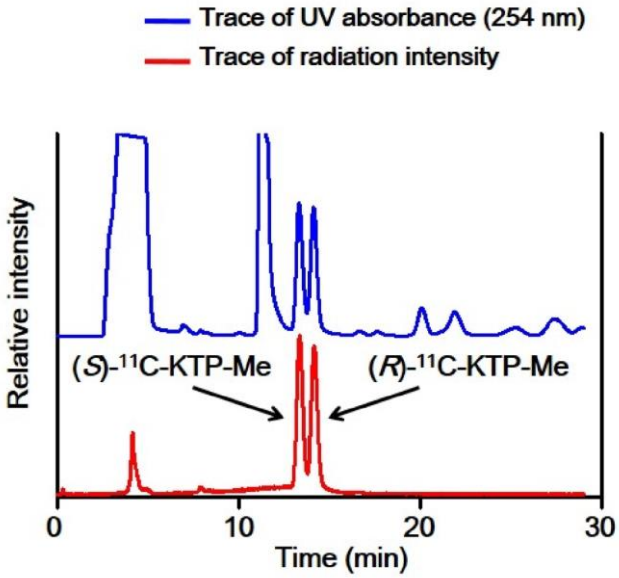
The reaction mixture containing (*RS*)-[^{11}C]KTP-Me was injected into a semi-preparative HPLC system with a chiral column to obtain (*R*)-[^{11}C]KTP-Me (mobile phase: acetonitrile/water = 52:48; column: CHIRALPAK, AS-RH, 20 mm i.d. × 150 mm, 5 μm; flow rate: 8.0 mL/min [0–1 min] to 10.0 mL/min [2–30 min]; UV detection: 254 nm; retention time of (*R*)-[^{11}C]KTP-Me: 15.8 min). The desired fraction was collected into a flask containing a pharmaceutical solution of 25% aqueous ascorbic acid and then concentrated under reduced pressure. The desired radiotracer was dissolved in a mixture (2 mL) of polysorbate 80, propylene glycol, and saline (0.5:10:100 v/v/v). The total synthesis time, including synthesis of (*RS*)-[^{11}C]KTP-Me, chiral resolution by HPLC, and

radiopharmaceutical formulation was less than 30 min. The [^{11}C]CH_3I-based decay-corrected radiochemical yield was calculated to be 20%. The chemical identity of (*R*)-[^{11}C]KTP-Me was confirmed by co-injection with an authentic sample of (*R*)-ketoprofen methyl ester using analytical HPLC (method 1; mobile phase: acetonitrile/water = 50:50; column: COSMOSIL, 5C_{18}-AR-II, 4.6 mm i.d. × 100 mm, 5 μm; flow rate: 1.0 mL/min; UV detection: 255 nm; retention time: 7.5 min, method 2; mobile phase: acetonitrile/water = 45:55; column: CHIRALPAK, AS-RH, 4.6 mm i.d. × 150 mm, 5 μm; flow rate: 1.0 mL/min; UV detection: 255 nm; retention time: 10.6 min). The chemical purity as determined by UV-HPLC analysis at 255 nm was greater than 99%, the radiochemical purity as determined by radio-HPLC analysis was greater than 99%, and the enantiomeric excess as determined by the chiral HPLC method was determined to be greater than 99%.

Separation of (*S*)-[^{11}C]KTP-Me with the chiral HPLC method

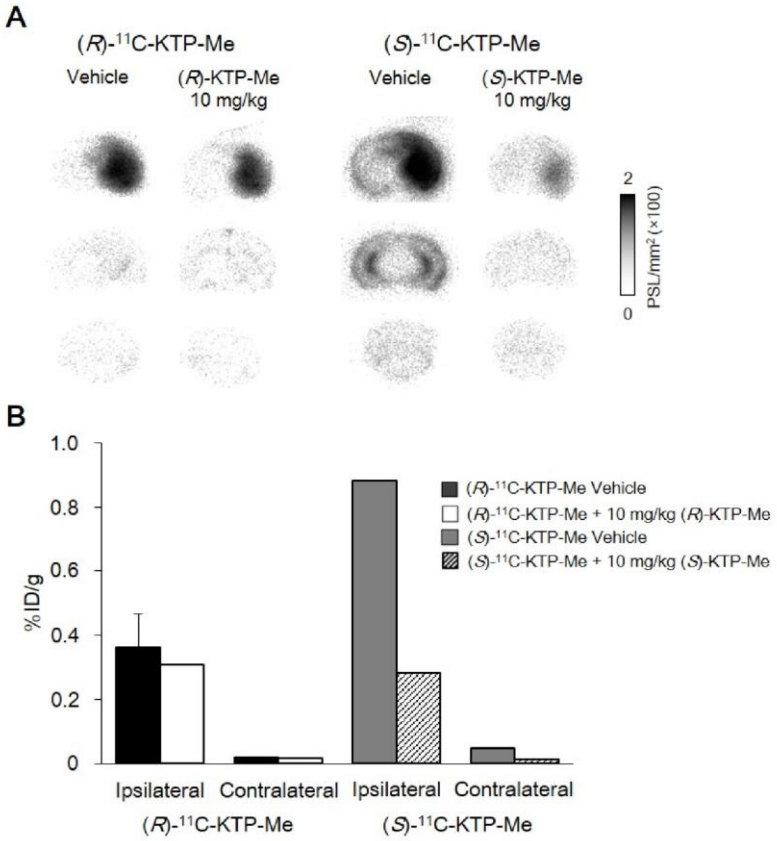
The reaction mixture containing (*RS*)-[^{11}C]KTP-Me was injected into a semi-preparative HPLC system with a chiral column to obtain (*S*)-[^{11}C]KTP-Me (mobile phase: acetonitrile/water = 50:50; column: CHIRALPAK, AS-RH, 20 mm i.d. × 150 mm, 5 μm; flow rate: 8.0 mL/min [0–1 min] to 10.0 mL/min [2–30 min]; UV detection: 254 nm; retention time of (*S*)-[^{11}C]KTP-Me: 13.4 min). The desired fraction was collected into a flask

containing a pharmaceutical solution of 25% aqueous ascorbic acid and then concentrated under reduced pressure. The desired radiotracer was dissolved in a mixture (2 mL) of polysorbate 80, propylene glycol, and saline (0.5:10:100 v/v/v). The total synthesis time, including synthesis of (*RS*)-[^{11}C]KTP-Me, chiral resolution of (*S*)-[^{11}C]KTP-Me by HPLC, and radiopharmaceutical formulation, was less than 30 min. The chemical identity of (*S*)-[^{11}C]KTP-Me was confirmed by co-injection with an authentic sample of (*S*)-ketoprofen methyl ester using analytical HPLC (method 1; mobile phase: acetonitrile/water = 53:47; column: COSMOSIL, 5C_{18}-AR-II, 4.6 mm i.d. × 100 mm, 5 μm; flow rate: 1.0 mL/min; UV detection: 255 nm; retention time: 5.9 min, method 2; mobile phase: acetonitrile/water = 50:50; column: CHIRALPAK, AS-RH, 4.6 mm i.d. × 150 mm, 5 μm; flow rate: 1.0 mL/min; UV detection: 255 nm; retention time: 6.2 min). The chemical purity as determined by UV-HPLC analysis at 255 nm was greater than 99%, the radiochemical purity as determined by radio-HPLC analysis was greater than 99%, and the enantiomeric excess as determined by the chiral HPLC method was determined to be greater than 99%.



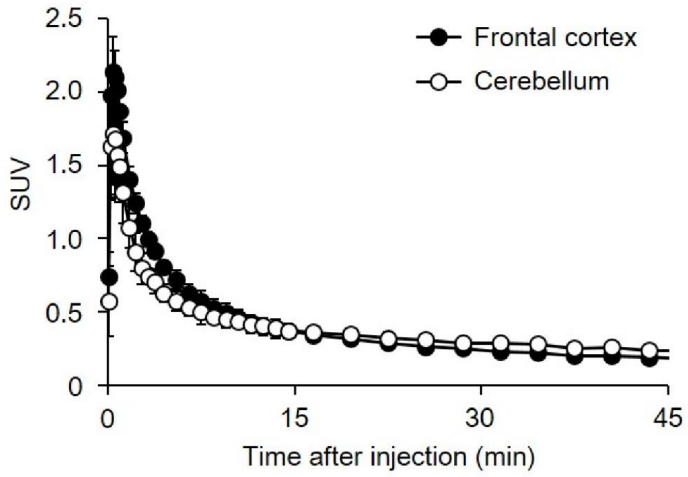
SUPPLEMENTAL FIGURE 1.

Separation of (*RS*)-^{11}C-KTP-Me into (*R*)- and (*S*)-^{11}C-KTP-Me. By using semi-preparative HPLC system with a chiral column (CHIRALPAK AS-RH, Daicel Co.), the reaction mixture containing (*RS*)-^{11}C-KTP-Me was separated into (*R*)- and (*S*)-^{11}C-KTP-Me, which were detected by both UV- (blue line) and radio-detectors (red line).



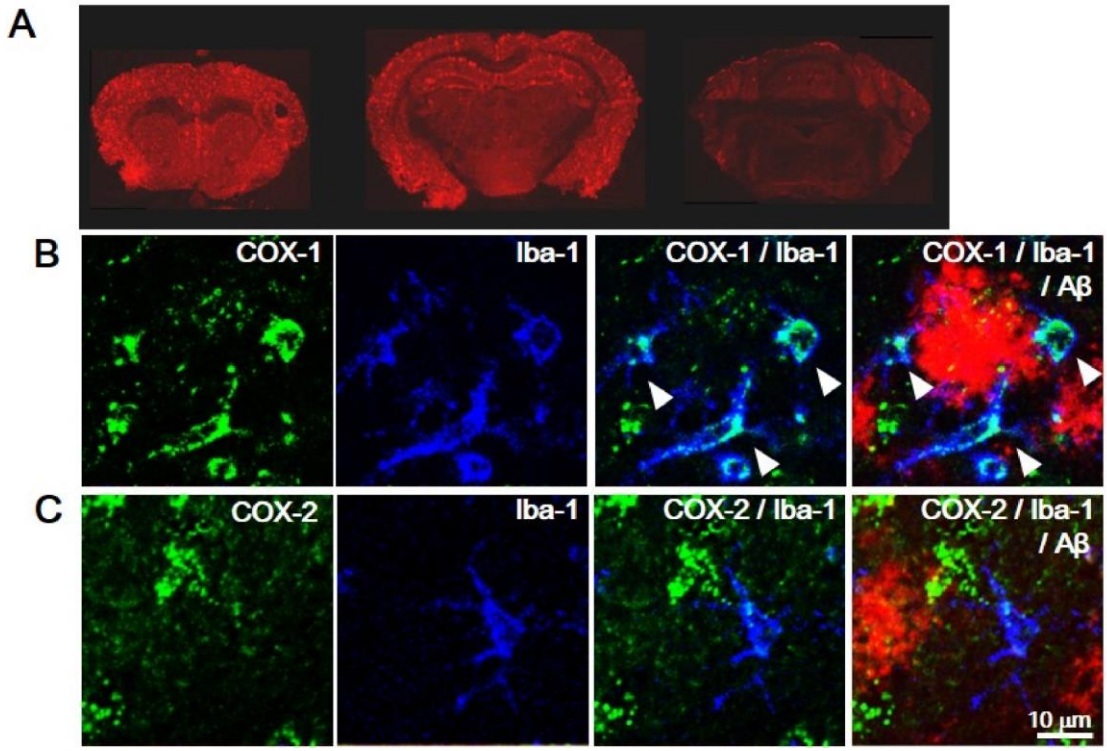
SUPPLEMENTAL FIGURE 2.

Displacement of (*R*)-^{11}C-KTP-Me and (*S*)-^{11}C-KTP-Me with unlabeled ligands. ***A***, Representative *ex vivo* autoradiographs of coronal sections of rat brain at 45 min after tracer injection. ***B***, Quantification of radioactivity in the LPS-injected ipsilateral and contralateral striatum. Unlabeled (*R*)-ketoprofen methyl ester and (*S*)-ketoprofen methyl ester (10 mg/kg) were simultaneously administered with (*R*)-^{11}C-KTP-Me and (*S*)-^{11}C-KTP-Me, respectively. Data are expressed as the percent injected dose/g and are the mean ± SD ((*R*)-^{11}C-KTP-Me-Vehicle, n = 3; (*R*)-^{11}C-KTP-Me-10 mg/kg (*R*)-KTP-Me, n = 1; Others, n = 2).



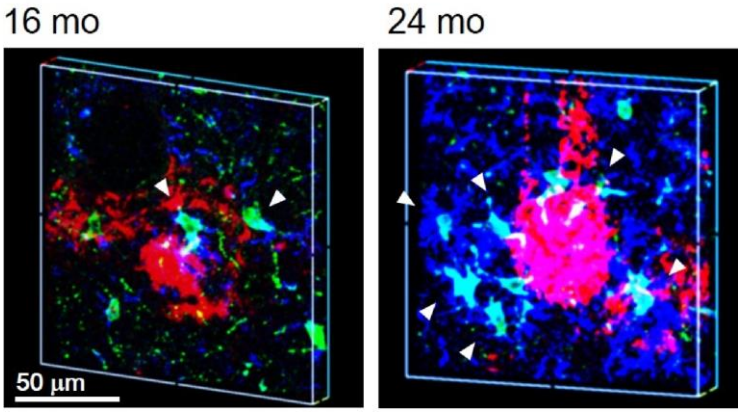
SUPPLEMENTAL FIGURE 3.

Time-activity curves of (*S*)-^{11}C-KTP-Me in mouse brain. Quantification of radioactivity in PET dynamic images of (*S*)-^{11}C-KTP-Me in wild-type mice (24 months old). Data are expressed as standard uptake value (SUV) and are the mean ± SD (n = 3).



SUPPLEMENTAL FIGURE 4.

Expression of COX-1 and COX-2 in microglia surrounding amyloid plaques in the APP-Tg 2576 mouse brain. ***A,*** Representative photomicrographs of immunofluorescent labeling for $A\beta_{1\text{-}16}$ in the brain of APP-Tg mice at 24 months old. ***B***, Representative photomicrographs of triple immunofluorescent labeling for COX-1 (green), Iba-1 (blue), and $A\beta_{1\text{-}16}$ (red) in the hippocampus of APP-Tg mice at 24 months old. COX-1 is expressed in Iba-1-positive microglia surrounding Aβ oligomers as indicated by arrows. ***C***, Representative photomicrographs of triple immunofluorescent labeling for COX-2 (green), Iba-1 (blue), and $A\beta_{1\text{-}16}$ (red) in the hippocampus of APP-Tg mice at 24 months old. COX-2 and Iba-1 were not co-expressed around Aβ oligomers.



SUPPLEMENTAL FIGURE 5.

Three-dimensional images of COX-1 (green), CD11b (blue), and $A\beta_{1\text{-}16}$ (red) in the hippocampus of APP-Tg mice at 16 and 24 months old. Arrows indicate cells co-expressing COX-1 and CD11b. COX-1-expressing activated microglia tightly surrounded and enclosed Aβ plaques were more abundant at 24 months rather than 16 months old mice.