Published in final edited form as:

Nucl Med Biol. 2007 February ; 34(2): 153–163. doi:10.1016/j.nucmedbio.2006.11.004.

Synthesis and positron emission tomography studies of carbon-11-labeled imatinib (Gleevec)

Kun-Eek Kil^{a,b}, Yu-Shin Ding^c, Kuo-Shyan Lin^d, David Alexoff^a, Sung Won Kim^{a,b}, Colleen Shea^a, Youwen Xu^a, Lisa Muench^a, and Joanna S. Fowler^{a,b,*}

^a Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA

^b Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794-3400, USA

^c Department of Radiology, Yale University School of Medicine, New Haven, CT 06520-8048, USA

^d Department of Radiology, University of Pittsburgh, Pittsburgh, PA 15213, USA

Abstract

Introduction—Imatinib mesylate (Gleevec) is a well known drug for treating chronic myeloid leukemia and gastrointestinal stromal tumors. Its active ingredient, imatinib ([4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridyl)-2-pyrimidinyl]amino]phenyl]benzamide), blocks the activity of several tyrosine kinases. Here we labeled imatinib with carbon-11 as a tool for determining the drug distribution and pharmacokinetics of imatinib, and we carried out positron emission tomography (PET) studies in baboons.

Methods—[N-¹¹C-methyl]imatinib was synthesized from [¹¹C]methyl iodide and norimatinib was synthesized by the demethylation of imatinib (isolated from Gleevec tablets) according to a patent procedure [Collins JM, Klecker RW Jr, Anderson LW. Imaging of drug accumulation as a guide to antitumor therapy. US Patent 20030198594A1, 2003]. Norimatinib was also synthesized from the corresponding amine and acid. PET studies were carried out in three baboons to measure pharmacokinetics in the brain and peripheral organs and to determine the effect of a therapeutic dose of imatinib. Log *D* and plasma protein binding were also measured.

Results—[N-¹¹C-methyl]imatinib uptake in the brain is negligible (consistent with P-glycoprotein-mediated efflux); it peaks and clears rapidly from the heart, lungs and spleen. Peak uptake and clearance occur more slowly in the liver and kidneys, followed by accumulation in the gallbladder and urinary bladder. Pretreatment with imatinib did not change uptake in the heart, lungs, kidneys and spleen, and increased uptake in the liver and gallbladder.

Conclusions—[N-¹¹C-methyl]imatinib has potential for assessing the regional distribution and kinetics of imatinib in the human body to determine whether the drug targets tumors and to identify other organs to which the drug or its labeled metabolites distribute. Paired with tracers such as 2-deoxy-2-[¹⁸F]fluoro-D-glucose (¹⁸FDG) and 3'-deoxy-3'-[¹⁸F]fluorothymidine (¹⁸FLT), [N-¹¹C-methyl]imatinib may be a useful radiotracer for planning chemotherapy, for monitoring response to treatment and for assessing the role of drug pharmacokinetics in drug resistance.

Keywords

Imatinib (Gleevec); PET; Carbon-11; Drug pharmacokinetics

*Corresponding author. Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA. Tel.: +1 631 344 4365; fax: +1 631 344 5815. fowler@bnl.gov (J.S. Fowler).

1. Introduction

Positron emission tomography (PET), coupled with a radiolabeled drug, is a powerful tool for determining drug distribution and pharmacokinetics [1]. This information would be of major importance in determining whether a chemotherapeutic drug targets a tumor and in determining other organs where the drug or its metabolites accumulate [2]. In addition, a study design in which a labeled chemotherapeutic drug is paired with a functional radiotracer such as ^{18}F FDG or ^{18}F FLT offers the potential to plan and monitor therapy and to make changes depending on individual response.

Imatinib mesylate (Gleevec; Fig. 1) ([4-[(4-methyl-1-piperazinyl)methyl]-*N*-[4-methyl-3-[[4-(3-pyridyl)-2-pyrimidinyl]amino]phenyl]benzamide methanesulfonate; STI571) is a member of a new class of signal transduction inhibitors used to treat chronic myeloid leukemia (CML) and gastrointestinal stromal tumor (GIST). It was the first drug to be rationally designed based on a molecular abnormality in CML and to be approved by the Food and Drug Administration in 2001 after remarkable success in the treatment of chronic-phase CML patients [3,4]. In fact, PET studies with ^{18}F FDG have documented a dramatic reduction in ^{18}F FDG uptake after the oral administration of 300–400 mg/day imatinib mesylate in GIST patients [5,6].

CML is a myeloproliferative disorder [7] triggered by genetic translocation between chromosome 9 and chromosome 22, producing aberrant Philadelphia chromosome [8,9]. This chromosome expresses the abnormal protein enzyme bcr-abl tyrosine kinase. Bcr-abl tyrosine kinase is constitutively altered so that it is no longer dependent on normal signal transduction induced by the interaction between cytokine (interleukin-3) and its receptor. Therefore, hematopoietic stem cells comprising bcr-abl tyrosine kinase exert enhanced cell function to produce white blood cells and blasts, as well as cell proliferation. For these reasons, bcr-abl tyrosine kinase serves as a good molecular target for CML treatment.

Imatinib, the active ingredient of Gleevec, was originally designed as a competitive inhibitor of bcr-abl tyrosine kinase in leukemic cells and of c-abl tyrosine kinase in normal cells (IC_{50} = 0.025 μM for both cells). Since c-abl tyrosine kinase does not play an important role in the cell survival of normal cells, imatinib spares normal cells while killing leukemic cells [10–12]. However, in spite of its efficacy, treatment resistance emerges due to the mutation of bcr-abl tyrosine kinase, which interferes with drug binding [13,14].

Imatinib also blocks other tyrosine kinases such as c-kit and platelet-derived growth factor (PDGF) [10,15] [IC_{50} = 0.41 μM (c-kit), IC_{50} = 0.38 μM (PDGF)]. c-kit contributes to the unique pathology of GIST [16], which is initiated in interstitial cells of Cajal, which play an essential role in intestinal motility [17]. c-kit undergoes a gain-of-function mutation in GIST [18–20]. Imatinib reduces tumor size in a significant fraction of GIST patients [21]. Unfortunately, similar to CML, resistance against imatinib also occurs in GIST by a secondary mutation. From this perspective, we reasoned that [*N*- ^{11}C -methyl]imatinib may be useful in determining whether drug pharmacokinetics changes when drug resistance develops.

In addition to chemotherapeutic applications for CML and GIST, preclinical studies show that imatinib has some potency against the deposition and accumulation of amyloid β -peptide, a characteristic peptide plaque found in the brain of Alzheimer's disease patients although blood–brain barrier penetration is a limitation [22,23]. Other conditions where imatinib shows promise are hepatocellular carcinoma [24,25], liver fibrosis [26,27] and pulmonary fibrosis [28,29], which are characterized by the expression of abl kinase, c-kit kinase or PDGF kinase.

Here we synthesized [*N*- ^{11}C -methyl]imatinib according to a recent patent procedure in which a nor precursor was prepared via the demethylation of imatinib [30]. We also prepared norimatinib from the corresponding amine and acid. We measured the distribution of [*N*- ^{11}C -

methyl]imatinib and/or its labeled metabolites in the brain and in peripheral organs in baboons at tracer doses and after treatment with a single dose of imatinib. This information is of relevance in determining the organs targeted by imatinib and its labeled metabolites.

2. Materials and methods

2.1. General

All chemicals used in synthesis were purchased from Sigma Aldrich Chemical Co. (Milwaukee, WI) and were used without any further purification. ^1H nuclear magnetic resonance (NMR) spectra were obtained in CDCl_3 solution (unless specified) using Bruker Avance 400 MHz NMR spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C) (Bruker Instruments, Inc., Billerica, MA) and were reported in parts per million downfield from tetramethylsilane as internal standard. Melting points were measured by Fisher–Johns melting point apparatus (Fisher Scientific Co., Pittsburgh, PA). High-resolution mass spectrometry (HRMS) experiments were obtained by the VG 7070 high-resolution mass spectrometer at the UCR Mass Spectrometry Facility (Riverside, CA). All reactions were monitored by analytical thin-layer chromatography (TLC). Spots were detected using UV light (254 nm) and, if appropriate, by 0.1% ninhydrin solution in isopropanol. An unlabelled standard of imatinib mesylate was generously provided by Dr. Brian J. Druker and was recovered from capsules.

2.2. Chemistry

2.2.1. Norimatinib synthesis from Gleevec capsules

2.2.1.1. Recovering imatinib free base from Gleevec capsules: A 100-mg Gleevec capsule contains 100 mg of imatinib and 218.5 mg of inert ingredients [31]. To recover imatinib, the cover of one capsule of Gleevec was removed, and powder was dissolved in 3 ml of distilled water. The suspension was filtered and washed with 2 ml of distilled water. Sodium bicarbonate aqueous solution (0.2 M, 10 ml) was added to the filtrate drop by drop until it was fully suspended. After the solution had been crystallized overnight, it was filtered. For further purification, the solid was recrystallized from methylene chloride–hexane cosolvent. The product was collected by filtration and dried in vacuo to yield 0.0879 g of imatinib (**1**). Yield=87.9%, *m.p.* =208–209°C (literature, 207–212°C) [32,33]. NMR was consistent with previous literature [33].

2.2.1.2. Preparation of 4-[(piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridyl)-2-pyrimidinyl] amino]phenyl benzamide (2) from imatinib free base (1): Norimatinib (**2**) (0.067 g) was obtained from imatinib (0.20 g, 0.4052 mmol) by nonclassical Polonovski demethylation [34], according to the procedure detailed in a US patent [30]. Yield=34.5%, *m.p.* =145–149°C. ^1H NMR: δ =2.36 (s, 3H), 2.46 (s, 4H), 2.91–2.94 (t, J =4.8 Hz, 4H), 3.56 (s, 2H), 3.58 (s, ^1H), 7.06 (s, ^1H), 7.19–7.20 (d, J =3.6 Hz, ^1H), 7.21–7.23 (d, J =8.3 Hz, ^1H), 7.30–7.33 (dd, J =8.1 Hz, 1.9 Hz, ^1H), 7.42–7.47 (m, 3H), 7.84–7.86 (d, J =8.2 Hz, 2H), 7.90 (s, ^1H), 8.52–8.53 (d, J =5.2 Hz, ^1H), 8.60 (d, J =1.3 Hz, ^1H), 8.70–8.72 (dd, J =4.8 Hz, 1.6 Hz, ^1H), 9.25–9.26 (d, J =1.5 Hz, ^1H). ^{13}C NMR ($\text{DMSO}-d_6$): δ =165.27, 161.59, 161.18, 159.47, 151.38, 148.19, 142.11, 137.78, 137.21, 134.41, 133.70, 132.20, 130.01, 128.66, 127.58, 128.52, 123.78, 117.19, 116.71, 107.50, 62.41, 54.14, 45.58, 17.65.

2.2.2. Preparation of N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine for norimatinib and imatinib

2.2.2.1. 3-(N,N-dimethylamino)-1-(3-pyridyl)-2-propen-1-one (3): The procedure used to synthesize β -(dimethylamino)vinyl 2-pyridyl ketone was adapted to synthesize 3-(N,N-dimethylamino)-1-(3-pyridyl)-2-propen-1-one (**3**) [35]. Yield=75.9%. ^1H NMR: δ =2.91 (s, 3H), 3.13 (s, 3H), 5.62–5.65 (d, J =12 Hz, ^1H), 7.29–7.33 (qd, J =4.4 Hz, 1.0 Hz, ^1H), 7.78–

7.81 (d, $J=12$ Hz, ^1H), 8.13–8.16 (dt, $J=8$ Hz, 1.2 Hz, ^1H), 8.61–8.63 (dd, $J=5.2$ Hz, 1.6 Hz, ^1H), 9.04–9.05 (dd, $J=2.4$ Hz, 0.7 Hz, ^1H). ^{13}C NMR: $\delta = 186.74, 155.08, 151.85, 149.30, 136.03, 135.46, 123.68, 92.21, 45.61, 37.78$.

2.2.2.2. 2-Methyl-5-nitrophenyl-guanidine nitrate (4): The procedure for the synthesis of 3-nitrophenyl-guanidine nitrate was adapted to synthesize 2-methyl-5-nitrophenyl-guanidine nitrate (**4**) (4.114 g) from 4.61 g of 2-methyl-5-nitroaniline [36]. Yield=56.3%, *m.p.* =218–219°C (literature, 214–220°C). NMR was consistent with previous literature [33].

2.2.2.3. N-(2-methyl-5-nitrophenyl)-4-(3-pyridyl)-2-pyrimidinamine (5): Phenylamino-pyrimidine (**5**) was prepared from enaminone (**3**) and guanidine derivative (**4**) according to the procedure detailed in Zimmerman et al. [37]. Yield=55.6%, *m.p.* =194–195°C (literature, 193–198°C). NMR was consistent with previous literature [33].

2.2.2.4. N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidinamine (6): Pyrimidinamine (**6**) was prepared by the reduction of nitro group in phenylamino-pyrimidine (**5**) using a modification of the procedure by Satoh et al. [38]. Yield=71.8%, *m.p.* =137–139°C (literature, 141–144°C) [32]. NMR was consistent with previous literature [33].

2.2.3. Benzyl 4-(4'-hydroxycarbonylbenzyl)-1-piperazine carboxylate (7)—The procedure of Metcalf et al. [39] was adapted to synthesize benzyl 4-(4'-hydroxy-carbonyl-benzyl)-1-piperazine carboxylate (**7**) from 4-bromomethyl benzoic acid and benzyl 1-piperazine-carboxylate. Yield=39.4%, *m.p.* =180–182°C. ^1H NMR (DMSO- d_6): $\delta = 2.33\text{--}2.36$ (t, $J=5.2$ Hz, 4H), 3.40 (s, 4H), 3.55 (s, 2H), 5.07 (s, 2H), 7.29–7.39 (m, 5H), 7.42–7.44 (d, $J=7.6$ Hz, 2H), 7.89–7.91 (d, $J=7.6$ Hz, 2H), 12.85 (s, ^1H). ^{13}C NMR (DMSO- d_6): $\delta = 167.27, 154.40, 143.13, 136.92, 129.79, 129.31, 128.83, 128.44, 127.86, 127.56, 66.19, 61.46, 52.31, 43.48$. HRMS (DCI/NH $_3$): m/z for $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_4$ (MH^+)=355.1652 (calculated) and 355.1672 (found).

2.2.4. 4-(4'-Hydroxycarbonylbenzyl)-1-methyl-piperazine (9)—Piperazinomethyl benzoic acid (**9**) was prepared from α -bromo-*p*-tolunitrile and 1-methylpiperazine via piperazino-methyl benzonitrile (**8**) by the procedure detailed in previous literature [40]. Yield=71% (**8**) and 49% (**9**); *m.p.* =66–67°C (**8**) (literature, 66–68°C) [40]. ^1H NMR (**9**) (D_2O): $\delta = 3.03$ (s, 3H), 3.66 (broad s, 8H), 4.54 (s, 2H), 7.64–7.66 (d, $J=6.2$ Hz, 2H), 8.11–8.13 (d, $J=6.2$ Hz, 2H). ^{13}C NMR (**9**) (D_2O): $\delta = 169.91, 132.91, 131.84, 131.40, 130.43, 59.82, 50.25, 48.35, 42.74$.

2.2.5. Preparation of imatinib (1) and its derivative (4-[(4-benzoyloxycarbonyl-1-piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridyl)-2-pyrimidinyl]amino]phenyl benzamide (10)) by amide bond formation—Imatinib (**1**) and its derivative (**10**) were prepared by amide bond formation with pyrimidinamine (**6**) and their corresponding acids (**9**) and (**7**), respectively] using a modification of the procedure of Lee et al. [41]. Yield=41% (**1**) and 54% (**10**); *m.p.* =208°C (**1**) and 160–162°C (**10**). ^1H NMR (**10**): $\delta = 2.37$ (s, 3H), 2.43 (s, 4H), 3.52–3.55 (t, $J=5.2$ Hz, 4H), 3.58 (s, 2H), 5.14 (s, 2H), 7.10 (s, ^1H), 7.20–7.21 (d, $J=5.2$ Hz, ^1H), 7.22–7.24 (d, $J=8.0$ Hz, ^1H), 7.31–7.38 (m, 6H), 7.43–7.47 (m, 3H), 7.84–7.87 (d, $J=7.2$ Hz, 2H), 7.87 (s, ^1H), 8.52–8.55 (m, 2H), 8.59–8.60 (d, $J=2.4$ Hz, ^1H), 8.71–8.73 (dd, $J=4.8$ Hz, 2.8 Hz, ^1H), 9.27 (dd, $J=1.6$ Hz, 0.8 Hz, ^1H). ^{13}C NMR (**10**): $\delta = 165.53, 163.01, 160.77, 159.23, 155.46, 151.66, 148.71, 142.23, 138.03, 136.92, 136.74, 135.23, 134.34, 132.92, 131.04, 129.54, 128.71, 128.24, 128.11, 127.31, 124.48, 123.98, 115.51, 113.30, 108.62, 67.35, 62.71, 53.01, 44.00, 17.92$. HRMS (**10**) (Fast Atom Bombardment FAB): m/z for $\text{C}_{36}\text{H}_{36}\text{N}_7\text{O}_3$ (MH^+) = 614.2874 (calculated) and 614.2879 (found).

2.2.6. Preparation of norimatinib by deprotection reaction (4-[(piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridyl)-2-pyrimidinyl]amino]phenyl benzamide (2))—Norimatinib (2) was prepared from (10) by a modified procedure from the literature [42]. Yield = 40.9%, *m.p.* = 145–149°C.

2.3. Radiolabeling

[*N*-¹¹C-methyl]imatinib hydrochloride (1) (Fig. 2) was prepared by modifying the method published in a US patent [30]. [¹¹C]Methyl iodide was generated from GE PETtrace MeI Microlab and transferred into a long-neck V-shaped vessel containing 0.2 mg of (2) in 0.20 ml of dimethyl sulfoxide. When carbon-11 radioactivity peaked in the reaction vessel, as determined by NaI detector, the reaction vessel was sealed and heated at 80°C for 10 min in an oil bath. The reaction mixture was diluted with 1 ml of 0.06 N HCl solution, and the product was eluted with 27% acetonitrile/73% ammonium formate (0.1 M) cosolvent at a flow rate of 4.5 ml/min on a Phenomenex Luna C₁₈ semipreparative column (250×10 mm, 5 μm) with the Knauer high-performance liquid chromatography (HPLC) system (Sonntek, Inc., Woodcliff Lake, NJ) equipped with a model K-500 pump, a model 87 variable wavelength monitor (UV, 254 nm), a NaI radioactivity detector and two Hewlett-Packard 3390A integrators. Norimatinib eluted around 14.5–18.5 min. The product was collected around 18.8–23.1 min after injection and transferred to a rotary evaporator preloaded with 50 μl of 1 N HCl to coevaporate with acetonitrile. The residue was dissolved in 4 ml of 5% ethyl alcohol solution in saline and passed through a 0.22-μm Millipore filter (Millipore Corp., Billerica, MA) into a sterile vial for PET study. The synthesis time was 1 h from the end of cyclotron bombardment (EOB) to delivery. The radiochemical yield was 76.7±6.9% (*n* = 6) based on total [¹¹C]methyl iodide activity. Specific activity ranged from 1.1 to 1.4 Ci/μmol at the EOB and was determined as the ratio of the total carbon-11 eluted with the product peak to the mass of imatinib determined from the area under the HPLC peak referred to as standard curve.

2.4. Quality control of collected [*N*-¹¹C-methyl]imatinib hydrochloride

The radiochemical purity of purified [*N*-¹¹C-methyl]-imatinib hydrochloride was determined by both analytical HPLC system and TLC. An aliquot of the [¹¹C] product (10 μl) was eluted with 35% acetonitrile/65% ammonium formate (0.1 M) solution at a flow rate of 1.0 ml/min in the analytical HPLC system to check radiochemical purity. Its retention time was 6.1±1.6 min, and the radiochemical purity was >98%, as determined by comparing the radioactivity in the peak to the total carbon-11 injected. The product aliquot (15 μl) was coinjected with unlabeled imatinib free base standard solution (7 μl) and 0.3 N HCl (30 μl) to form one ionic species, labeled product and unlabeled standard coeluted (retention time=5.6±0.6 min).

TLC in which the labeled product and an authentic standard were cospotted also showed that the radiochemical purity was >98% and the *R_f* value was 0.48 (solvent: methanol/28–30% NH₄OH solution, 20:1, on Macherey–Nagel Polygram Sil G/UV254 plastic back TLC plate). The TLC plate was scanned by Bioscan System 200 Imaging Scanner (Bioscan, Inc., Washington DC). UV spot was checked with a 254-nm short-wave UV.

2.5. Determination of log *D*

Log *D* was determined by the modification of a procedure in the literature [43]. [*N*-¹¹C-methyl]imatinib hydrochloride solution (50 μl) was added to a test tube containing 2.5 ml of octanol and 2.5 ml of phosphate-buffered solution (pH 7.4). The solution was vortexed for 2 min and centrifuged for 2 min at maximum speed. An aliquot (0.1 μl) was taken from an organic layer, and another aliquot (1.0 μl) was taken from an aqueous layer. Then 2.0 ml of sample from the organic layer was added to a second test tube containing 0.5 ml of octanol and 2.5 ml of phosphate-buffered solution (pH 7.4). The same treatment is applied to the second test tube. These repeated procedures were applied to six test tubes so that 12 samples (six from the

aqueous layer and six from the organic layer) were obtained. Each sample was counted by a well counter (Picker, Cleveland, OH) to yield the ratio of decay-corrected counts in octanol to decay-corrected counts in buffer.

2.6. PET studies of [N-¹¹C-methyl]imatinib in baboons

The study was performed under the strict control of the NIH Guide for the Care and Use of Laboratory Animals (US Department of Health and Human Services) and was approved by the Brookhaven National Laboratory Institutional Animal Care and Use Committee. Three female baboons (*Papio anubis*) were prepared and anesthetized according to a procedure published previously [44]. An intramuscular injection of ketamine hydrochloride (10 mg/kg) was given, and the animal was intubated and transported to a PET facility in a temperature-controlled transfer cage. Oxygen (800 ml/min), nitrous oxide (1500 ml/min) and isoflurane (1–4%) were provided to keep the animals sedated. Catheters were inserted in an antecubital vein for radiotracer injection, and another line was inserted in the radial artery for blood sampling. Vital signs, such as heart rate, respiration rate, arterial partial pressure of oxygen and temperature, were monitored during the study. A transmission scan was performed with a ⁶⁸Ge rotating rod source before [N-¹¹C-methyl]imatinib was injected. Two intravenous injections of [N-¹¹C-methyl]imatinib were administered to each animal. In one of the animals, a dynamic brain scan and a dynamic torso (encompassing the liver and the kidneys) scan were carried out 2 h apart; in the second animal, two dynamic torso scans were performed at different positions to optimize the coverage of peripheral organs from the heart to the urinary bladder; in a third animal, two dynamic torso scans were performed (covering the heart to the kidneys) with an intervening therapeutic dose of free imatinib, 32 mg, iv (as imatinib hydrochloride, which is equivalent to a 400-mg oral dose of imatinib mesylate) [45], infused over a 60-min time period prior to the second tracer dose. Imatinib hydrochloride solution was synthesized according to the scheme in Fig. 2 by dissolving free imatinib (32 mg) in 1.95 ml of 0.1 N hydrochloric acid solution. The excess amount of hydrochloric acid was removed by coevaporation with acetonitrile to give imatinib hydrochloride salt, which was dissolved in 5 ml of sterile water and passed through a Millipore filter. Doses of [N-¹¹C-methyl]-imatinib ranged from 2.8 to 6.2 mCi (*n* = 6), and specific activity ranged from 0.1 to 0.13 Ci/μmol at the time of injection for the six PET studies.

Scanning was carried out for 90 min with a high-resolution PET (Siemens HR⁺; 63 slices; 4.5×4.5×4.5 mm at the center of the field of view) in 3D mode. Scanning sequences were following; 10 frames of 1 min, 4 frames of 5 min and 8 frames of 7.5 min, except one of the torso scans, which was terminated at 71 min. Arterial blood sampling was conducted every 2.5 s (OleDich blood sampling machine; Hvidovre, Denmark) for first 2.5 min, and then was obtained at 5, 10, 20, 30, 60 min and at the end of scanning. All samples were centrifuged to obtain plasma samples, whose radioactivity was measured in a well counter calibrated with a ⁶⁸Ge/⁶⁸Ga source. Plasma samples at 1, 5, 10, 30, 60 and 90 min were subjected to HPLC analysis to determine the fraction of carbon-11 present as parent radiotracer (Section 2.7).

2.7. HPLC determination of [N-¹¹C-methyl]imatinib fraction in plasma

Arterial plasma samples were added to 0.3 ml of acetonitrile containing 20 μl of standard unlabeled imatinib solution (1 mg/ml). After measuring total activity, each solution was homogenized for 10 s and centrifuged for 5 min. The resulting supernatant (*A*_{total}) was counted and injected into the analytical HPLC system [Phenomenex Luna C₁₈ analytical column (250×4.6 mm, 5 μm) with a UV detector (254 nm)] eluting with 35% acetonitrile/65% ammonium formate (0.1 M) solution at a flow rate of 1.0 ml/min. The fraction of unchanged parent radiotracer was determined as the ratio of counts in imatinib fraction (*A*) to the total carbon-11 injected onto the column (*A*_{total}): *A*/*A*_{total}.

2.8. Plasma protein binding of [N-¹¹C-methyl]imatinib

The free fraction of [N-¹¹C-methyl]imatinib in plasma was measured according to the procedure in the literature [46]. A diluted aliquot (10 μ l) of [N-¹¹C-methyl]imatinib was added to baboon plasma (0.8 ml) and incubated for 10 min at room temperature. An aliquot (20 μ l) was counted in a well counter (unspun aliquot). A portion of the sample (0.2 ml) was charged into the upper level of a Centrifree tube (Amicon, Inc., Beverly, MA) and centrifuged for 10 min. The top part of the Centrifree tube was removed, and an aliquot of the solution remaining in the bottom cup was counted in a well counter (unbound sample). The free fraction is the ratio of the decay-corrected counts of unbound aliquot to the decay-corrected counts of unspun aliquot.

2.9. Image analysis

Time frames were summed over the experimental period, and planes were summed in groups of two for region-of-interest placement. Regions of interest were placed over the brain and peripheral organs (heart, lungs, spleen, liver, gallbladder, kidneys, urinary bladder and spinal cord) and then projected onto dynamic images to obtain time–activity curves. Carbon-11 concentration in each region of interest was divided by the injected dose and multiplied by 100 to obtain percent dose per cc. For the pretreatment study, we compared the time–activity curves at baseline and after pretreatment. We also compared the time–activity curves for unchanged tracer in plasma and the area under the curve for the plasma at baseline and after treatment. For the pretreatment study, we also normalized the time–activity curves in each organ for the area under the plasma time–activity curve at each time point [% dose/cc/AUC(*t*)] to determine whether changes were driven by treatment-induced changes in plasma.

3. Results and discussion

3.1. Chemistry and carbon-11 labeling

Demethylation of imatinib with 3-chloroperbenzoic acid (*m*-CPBA) and iron sulfate by nonclassical Polonovski reaction according to the patent procedure gave a modest yield (34.5%) [30,34]. Norimatinib (**2**) and imatinib (**1**) were also synthesized from commercially available starting materials by adapting a series of literature methods [33] (Fig. 2). Norimatinib was labeled successfully at 80°C for 10 min without any base by modifying the labeling scheme in a published patent [30] (Fig. 2). [N-¹¹C-methyl]imatinib was obtained in 76.7 $\pm$ 6.9% (*n*=6) yield based on total [¹¹C]methyl iodide activity. The radiochemical purity determined by TLC and HPLC analytical system was >98%. Specific activity ranged from 1.1 to 1.4 Ci/ μ mol at the EOB, and the synthesis time was 1 h from EOB. The experimental value of log *D* was 2.34 $\pm$ 0.17.

3.2. Evaluation of [N-¹¹C-methyl]imatinib with PET

As can be seen in the summed PET images of baboon brain after the injection of 3.9 mCi of [N-¹¹C-methyl]imatinib, carbon-11 was not taken into the brain but was concentrated in sinuses and tissues surrounding the brain (Fig. 3). Even though imatinib satisfies Lipinski's rule of five [47] with a log *D* of 2.34 $\pm$ 0.17, which is ideal for blood–brain barrier penetration, and with 11.4% unbound in plasma, its brain distribution is known to be limited by P-glycoprotein-mediated efflux [48,49]. P-glycoprotein-mediated efflux contributes not only to blood–brain barrier elimination of imatinib but also to the development of imatinib resistance [50]. A summed PET image of baboon torso showed that [N-¹¹C-methyl]imatinib and/or its labeled metabolites concentrated mainly in the liver, kidneys and gallbladder after intravenous injection (Fig. 4).

The time–activity curves for these organs are shown in Fig. 5A and B for two baboons in which four scans were performed to cover the entire torso from the brain to the urinary bladder. We found that [*N*-¹¹C-methyl]imatinib and/or its labeled metabolites peaked early in the heart, lungs and spleen and cleared rapidly, while the liver and kidney peaked and cleared more slowly, and gallbladder accumulated carbon-11 over the time course of the study. Carbon-11 accumulation in the gallbladder may represent the excretion of imatinib or its labeled metabolite(s). We note that imatinib is mainly metabolized by the cytochrome *P*450 3A4–cytochrome *P*450 3A isoenzyme system [51–53]. The time–activity curve for the urinary bladder shows a transient large peak between 50 and 70 min, which is likely to represent urinary excretion (Fig. 5B). There was very little uptake of carbon-11 in the spinal cord (data not shown) and lungs. Thus, it may be possible to determine whether [*N*-¹¹C-methyl]imatinib accumulates in tumors occurring in these regions. [*N*-¹¹C-methyl]imatinib may be a useful tool for planning chemotherapy in GIST patients and in other conditions where imatinib therapy may be warranted.

Labeled imatinib and its labeled metabolites cleared rapidly from the plasma. Treatment of plasma samples with acetonitrile in preparation for HPLC analysis yielded 80% of the total carbon-11 in the supernatant. The percentage of unchanged imatinib as measured by HPLC was 93.8±3.9%, 85.1±6.2%, 79.6±2.9%, 64.6±6.8%, 47.9±17.3% at 1, 5, 10, 30 and 60 min, respectively (*n*=3).

3.3. [*N*-¹¹C-methyl]imatinib pharmacokinetics after pretreatment with a therapeutic dose of imatinib

Since imatinib is given at therapeutic doses in cancer treatment, we also compared the pharmacokinetics of [*N*-¹¹C-methyl]imatinib before and after an intravenous dose of 32 mg of imatinib (equivalent to a 400-mg typical oral chemotherapeutic dose scaled from a 70-kg human to a 15-kg baboon) [45].

After pretreatment, we found reduced uptake in the heart, lungs, kidneys and spleen at early times, whereas the time–activity curves for these organs paralleled one another after 40 min; in contrast, uptake was higher in the liver and gallbladder after imatinib treatment (Fig. 6). We also found a reduced concentration of [*N*-¹¹C-methyl]imatinib in the plasma after pretreatment (Fig. 7). However, when the time–activity curves for the heart, lungs, kidneys and spleen were normalized for plasma, there were no differences indicating that reductions in uptake are driven by reductions in plasma. In contrast, normalized time–activity curves in the liver and gallbladder showed increased carbon-11 uptake, which may reflect an increased accumulation of labeled metabolites (Fig. 6, insets). We note a recent report of imatinib-induced cardiotoxicity [54], which could result from the high exposure of heart tissues during chronic administration.

4. Conclusions

[*N*-¹¹C-methyl]imatinib has potential for assessing the regional distribution and kinetics of imatinib in the human body to determine whether the drug targets tumors and to identify other organs to which the drug or its labeled metabolites distribute. Paired with tracers such as ¹⁸F¹⁸FDG and ¹⁸F¹⁸FLT, [*N*-¹¹C-methyl]imatinib may be a useful radiotracer in planning chemotherapy, in monitoring response to treatment and in assessing the role of drug pharmacokinetics in drug resistance. Although the use of [*N*-¹¹C-methyl]imatinib as a biomarker for mutant tyrosine kinases may be possible, this would need to be validated in vivo preferably in cancer patients to determine whether carbon-11 uptake is associated with the presence of abnormal tyrosine kinases, whether uptake is saturable and whether pharmacokinetics changes with drug resistance.

Acknowledgments

This work was carried out at the Brookhaven National Laboratory under contract DE-AC02-98CH10886 with the US Department of Energy and supported by its Office of Biological and Environmental Research and by the National Institute on Drug Abuse (K05DA020001). The authors thank Michael Schueller and David Schlyer for the operation of cyclotron, Donald Warner for PET operations, Payton King and Pauline Carter for performance of baboon studies. The authors are also grateful to Michael Viola for bringing this problem to our attention, and to Nora D. Volkow and Bengt Langstrom for helpful discussions. They are also grateful to Dr. Brian J. Druker for providing a sample of Gleevec.

References

1. Fowler JS, Volkow ND, Wang G-J, Ding Y-S, Dewey S-L. PET and drug research and development. *J Nucl Med* 1999;40:1154–63. [PubMed: 10405137]
2. Collins, JM. PET and drug development. In: Wahl, RL.; Buchanan, JW., editors. Principles and practice of positron emission tomography. Philadelphia, PA: Lippincott Williams and Wilkins; 2002. p. 411–9.
3. Saglo G, Morotti A, Mattioli G, Messa E, Giugliano E, Volpe G, et al. Rational approaches to the design of therapeutics targeting molecular markers: the case of chronic myeloid leukemia. *Ann N Y Acad Sci* 2004;1028:423–31. [PubMed: 15650267]
4. Cohen P. Protein kinase — the major drug targets of the twenty-first century? *Nat Rev Drug Discov* 2002;1:309–15. [PubMed: 12120282]
5. Joensuu H. Treatment of inoperable gastrointestinal stromal tumor (GIST) with imatinib (Gleevec). *Med Klin (Munich)* 2002;97:S128–30.
6. Zaknun JJ, Kendler D, Moncayo R, zur Nedden D, Virgolini I. F-18 FDG PET for assessing tyrosine kinase-related signal transduction inhibition in a GIST c-kit-positive tumor patient by imatinib. *Clin Nucl Med* 2005;30:749–51. [PubMed: 16237304]
7. Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a therapeutic agent for chronic myeloid leukemia. *Blood* 2005;105:2640–53. [PubMed: 15618470]
8. Nowell P, Hungerford D. A minute chromosome in human chronic granulocytic leukemia. *Science* 1960;132:1497.
9. Rowley JD. A new consistent chromosomal abnormality in chronic myeloid leukemia identified by quinacrine fluorescence and Giemsa staining. *Nature* 1973;243:290–3. [PubMed: 4126434]
10. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the abl tyrosine kinase on the growth of bcr-abl positive cells. *Nat Med* 1996;2:561–6. [PubMed: 8616716]
11. Deininger M, Goldman GM, Lydon NB, Melo JV. The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of bcr-abl positive cells. *Blood* 1997;90:3691–8. [PubMed: 9345054]
12. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, et al. Crystal structure of the kinase domain of c-abl in complex with the small molecule inhibitors PD173955 and imatinib (STI-571). *Cancer Res* 2002;62:4236–43. [PubMed: 12154025]
13. La Rosee P, Corbin AS, Stoffregen EP, Deininger MW, Druker BJ. Activity of the bcr-abl kinase inhibitor PD180970 against clinically relevant bcr-abl isoforms that cause resistance to imatinib mesylate (Gleevec STI571). *Cancer Res* 2002;62:7149–53. [PubMed: 12499247]
14. Cowan-Jacob SW, Guez V, Fendrich G, Griffin JD, Fabbro D, Furet P, et al. Imatinib (STI571) resistance in chronic myelogenous leukemia: molecular basis of the underlying mechanisms and potential strategies for treatment. *Mini Rev Med Chem* 2004;4:285–99. [PubMed: 15032675]
15. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, et al. Abl protein-tyrosine kinase inhibitor STI571 inhibits in vitro signal transduction mediated by c-kit and platelet-derived growth factor receptors. *J Pharmacol Exp Ther* 2000;295:139–45. [PubMed: 10991971]
16. Tuveson DA, Willis NA, Jacks T, Griffin JD, Singer S, Fletcher CD, et al. STI571 inactivation of the gastrointestinal stromal tumor c-kit oncoprotein: biological and clinical implications. *Oncogene* 2001;20:5048–58.
17. Kindblom LG, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotype characteristics of the intestinal cells of Cajal. *Am J Pathol* 1998;152:1259–69. [PubMed: 9588894]

18. Hirota S, Isozaki K, Moritama Y, Hashimoto K, Nishida T, Ishiguro S, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998;279:577–80. [PubMed: 9438854]
19. Antonescu CR, Sommer G, Sarraf L, Tschernyavsky SJ, Riedel E, Woodruff JM, et al. Association of kit exon 9 mutations with nongastric primary site and aggressive behavior: kit mutation analysis and clinical correlates of 120 gastrointestinal stromal tumors. *Clin Cancer Res* 2003;9:3329–37. [PubMed: 12960119]
20. Lux ML, Rubin BP, Biase TL, Chen C-J, Maclure T, Demetri G, et al. Kit extracellular and kinase domain mutations in gastrointestinal stromal tumors. *Am J Pathol* 2000;156:791–5. [PubMed: 10702394]
21. Verweij J, Casali PG, Zalcberg J, LeCesne A, Reichardt P, Blay J-Y, et al. Progression-free survival in gastrointestinal stromal tumors with high-dose imatinib: randomized trial. *Lancet* 2004;364:1127–34. [PubMed: 15451219]
22. Netzer WJ, Dou F, Cai D, Veach D, Jean S, Li Y, et al. Gleevec inhibits β -amyloid production but not notch cleavage. *Proc Natl Acad Sci U S A* 2003;100:12444–9. [PubMed: 14523244]
23. Takayama N, Sato N, O'Brien SG, Ikeda Y, Okamoto S-I. Imatinib mesylate has limited activity against the central nervous system involvement of Philadelphia chromosome-positive acute lymphoblastic leukaemia due to poor penetration into cerebrospinal fluid. *Br J Haematol* 2002;119:106–8. [PubMed: 12358909]
24. Ramadori G, Füzesi L, Grabbe E, Pieler T, Armbrust T. Successful treatment of hepatocellular carcinoma with the tyrosine kinase inhibitor imatinib in a patient with liver cirrhosis. *Anticancer Drugs* 2004;15:405–9. [PubMed: 15057146]
25. Eckel F, von Delius S, Mayr M, Dobritz M, Fend F, Hosius C, et al. Pharmacokinetic and clinical phase II trial of imatinib in patients with impaired liver function and advanced hepatocellular carcinoma. *Oncology* 2005;69:363–71. [PubMed: 16319507]
26. Yoshiji H, Noguchi R, Kuriyama S, Ikenaka Y, Yoshii J, Yanase K, et al. Imatinib mesylate (STI-571) attenuates liver fibrosis development in rats. *Am J Physiol Gastrointest Liver Physiol* 2005;288:G907–13. [PubMed: 15618280]
27. Neef M, Ledermann M, Saegesser H, Schneider V, Widmer N, Decosterd LA, et al. Oral imatinib treatment reduces early fibro-genesis but does not prevent progression in the long term. *J Hepatol* 2006;44:167–75. [PubMed: 16168515]
28. Abdollahi A, Li M, Ping G, Plathow C, Domhan S, Kiessling F, et al. Inhibition of platelet-derived growth factor signaling attenuates pulmonary fibrosis. *J Exp Med* 2005;201:925–35. [PubMed: 15781583]
29. Aono Y, Nishioka Y, Inayama M, Ugai M, Kishi J, Uehara H, et al. Imatinib as a novel antifibrotic agent in bleomycin-induced pulmonary fibrosis in mice. *Am J Respir Crit Care Med* 2005;171:1279–85. [PubMed: 15735062]
30. Collins, JM.; Klecker, RW., Jr; Anderson, LW. Imaging of drug accumulation as a guide to antitumor therapy. US Patent. 20030198594A1. 2003.
31. Zimmermann, J.; Burger, HM. Crystal modification of a N-phenyl-2-pyrimidineamine derivative, process for its manufacture and its use. WO 9903854. 1999.
32. Zimmerman J, Buchdunger E, Mett H, Meyer T, Lydon NB. Potent and selective inhibitors of the abl-kinase: phenylamino-pyrimidine (PAP) derivatives. *Bioorg Med Chem Lett* 1997;7:187–92.
33. Szczepek, W.; Luniewski, W.; Kaczmarek, L.; Zagrodzki, B.; Samson-Lazinska, D.; Szelejewski, W., et al. A process for preparation of imatinib base. WO 2006071130 A2. 2006.
34. Mary A, Renko DZ, Guillou C, Thai C. Selective N-demethylation of galanthamine to norgalanthamine via a non classical Polonovski reaction. *Tetrahedron Lett* 1997;38:5151–2.
35. Jameson DL, Guise LE. An improved, two-step synthesis of 2,2':6',2''-terpyridine. *Tetrahedron Lett* 1991;32:1999–2002.
36. Zimmerman, J. Pyrimidine derivatives and processes for the preparation thereof. US Patent. 5,521,184. 1994.
37. Zimmerman J, Buchdunger E, Mett H, Meyer T, Lydon NB, Traxler P. Phenylamino-pyrimidine (PAP)-derivatives: a new class of potent and highly selective PDGF-receptor autophosphorylation inhibitors. *Bio-org Med Chem Lett* 1996;6:1221–6.

38. Satoh T, Suzuki S, Suzuki Y, Miyaji Y, Imai Z. Reduction of organic compounds with sodium borohydride-transition metal salt system: 1. Reduction of organic nitrile, nitro and amide compounds to primary amines. *Tetrahedron Lett* 1969;52:4555–8.
39. Metcalf, CA., III; Shakespeare, WC.; Sawyer, TK.; Wang, Y.; Bohacek, R. Preparation of phosphorus-substituted pyridopyrimidines as therapeutic agents. WO 2003000011. 2003.
40. Sairam P, Puranik R, Kelkar AS, Sasikiran S, Veerender M, Parvathi A. The ester and amide derivatives of 4-(4-methyl piperazinomethyl)benzoic acid. *Synth Commun* 2003;33:3597–605.
41. Lee CK, Yu JS, Ji YR. Determination of aromaticity indices of thiophene and furan by nuclear magnetic resonance spectroscopic analysis of their anilides. *J Heterocycl Chem* 2002;39:1219–27.
42. Barker PL, Gendler PL, Rapoport H. Acylation of dibasic compounds containing amino amidine and aminoguanidine functions. *J Org Chem* 1981;46:2455–65.
43. Del Rosario RB, Jung Y-W, Baidoo KE, Lever SZ, Wieland DM. Synthesis and in vivo evaluation of a $^{99m/99}\text{Tc}$ -DADT-benzovesamicol: a potential marker for cholinergic neurons. *Nucl Med Biol* 1994;21:197–203. [PubMed: 9234283]
44. Ding Y-S, Fowler JS, Volkow ND, Logan J, Gatley SJ, Sugano Y. Carbon-11-D-threo-methylphenidate binding to dopamine transporter in baboon brain. *J Nucl Med* 1995;36:2298–305. [PubMed: 8523123]
45. Peng B, Dutreix C, Mehring G, Hayes MJ, Ben-Am M, Seiberling M, et al. Absolute bioavailability of imatinib (Gleevec) orally versus intravenous infusion. *J Clin Pharmacol* 2004;44:158–62. [PubMed: 14747424]
46. Ding Y-S, Lin K-S, Logan J, Benveniste H, Carter P. Comparative evaluation of positron emission tomography radiotracers for imaging the norepinephrine transporter: (*S,S*) and (*R,R*) enantiomers of reboxetine analogs (^{11}C] methylreboxetine, 3-Cl- ^{11}C] methylreboxetine and ^{18}F] fluoreoreboxetine), (*R*)- ^{11}C] nioxetine, ^{11}C] oxaprotiline and ^{11}C] lortalamine. *J Neurochem* 2005;94:337–51. [PubMed: 15998285]
47. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 1997;23:3–25.
48. Dai HQ, Marbach P, Lemaire M, Hayes M, Elquist WF. Distribution of STI-571 to the brain is limited by P-glycoprotein-mediated efflux. *J Pharmacol Exp Ther* 2003;304:1085–92. [PubMed: 12604685]
49. Breedveld P, Pluim D, Cipriani G, Wielinga P, vanTellingen O, Schinkel AH, et al. The effect of bcrp1 (abcg2) on the in vivo pharmacokinetics and brain penetration of imatinib mesylate (Gleevec): implication for the use of breast cancer resistance protein and P-glycoprotein inhibitors to enable the brain penetration of imatinib in patients. *Cancer Res* 2005;65:2577–82. [PubMed: 15805252]
50. Mahon FX, Deininger MW, Schultheis B, Chabrol J, Reiffers J, Jgoldman M, et al. Selection and characterization of bcr-abl positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanism of resistance. *Blood* 2000;96:1070–9. [PubMed: 10910924]
51. NovartisPharmaStein AG M. Gleevec (imatinib mesylate): prescribing information [online]. [accessed October 24, 2006]. Available from URL: http://www.us.gleevec.com/hcp/page/hcp_prescribing_info
52. Cohen MH, Williams G, Johnson JR, Duan J, Gobburu J, Rahman A, et al. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. *Clin Cancer Res* 2000;8:935–42. [PubMed: 12006504]
53. Deinger MW, Druker BJ. Specific targeted therapy of chronic myelogenous leukemia with imatinib. *Pharmacol Rev* 2003;55:401–23. [PubMed: 12869662]
54. Kerkela R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med* 2006;12:908–16. [PubMed: 16862153]

Fig. 1.
Structure of imatinib (the active ingredient of Gleevec).

Fig. 2.
Synthetic scheme of norimatinib, imatinib and $[N-^{11}\text{C}\text{-methyl}]\text{imatinib}$.

Fig. 3. PET image of baboon brain with [*N*-¹¹C-methyl]imatinib. Summed frames over 90 min after the injection of 3.9 mCi of [*N*-¹¹C-methyl]imatinib showing lack of uptake of carbon-11 into the brain probably due to P-glycoprotein-mediated efflux [48,49]. The anesthesia used was ketamine, followed by isoflurane.

Fig. 4.

PET image of the torso of the anesthetized baboon with [*N*-¹¹C-methyl]imatinib. PET images (summation of frames over 90 min) of the baboon torso after the injection of 4.71 mCi of [*N*-¹¹C-methyl]imatinib showing the accumulation of carbon-11 in the heart and lungs (top row); in the liver and in the beginning of the gallbladder (second row); in the liver and gallbladder (third row); in the gallbladder and spleen (fourth row); and in the kidneys and spleen (fifth row). Note high accumulation in the gallbladder. The anesthesia used was ketamine, followed by isoflurane.

Fig. 5. Time-activity curves for two different anesthetized baboons (A and B) that each received two injections of $[N-^{11}\text{C-methyl}]$ imatinib in the brain through the urinary bladder.

Fig. 6. The comparison of time-activity curves of the (A) heart, (B) lungs, (C) kidneys, (D) spleen, (E) liver and (F) gallbladder before and after pretreatment with 32 mg of imatinib administration by intravenous injection. Time-activity curves normalized for the area under the plasma time-activity curve are shown in insets.

Fig. 7.

(A) Time-activity curve at baseline and after imatinib pretreatment for $[N-^{11}C\text{-methyl}]$ imatinib in plasma showing the first 5 min and (B) the plasma integral over 90 min. Pretreatment delayed $[N-^{11}C\text{-methyl}]$ imatinib peak and reduced its accumulated concentration in plasma by 10–15%.