Quality Control Procedure for 6-[^{18}F]Fluoro-L-DOPA: A Presynaptic PET Imaging Ligand for Brain Dopamine Neurons

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The goal of the current study was to establish a quality control procedure for clinical use of 6-[^{18}F]fluoro-L-DOPA (6-[^{18}F]-DOPA) as a selective presynaptic positron emission tomographic (PET) imaging ligand for brain dopamine neurons. A high performance liquid chromatographic procedure using a 5-μ C-18 reverse phase column and ion-pairing mobile phase was used for the quantification of 6-[^{18}F]-DOPA. The radiochemical purity of 6-[^{18}F]-DOPA was measured by ^{18}F radioactivity in HPLC fractions while the chemical purity was determined by an amperometric electrochemical detector with a sensitivity of 25 pg. Quality control of eight consecutive batches of highly purified 6-[^{18}F]-DOPA sample used in a pre-clinical trial revealed that the chemical and/or radiochemical purity of the PET imaging ligand, 6-[^{18}F]-DOPA was greater than 97 ± 0.5% with a specific activity of 365 ± 31 mCi/mmol. The knowledge and assurance of radiochemical purity of PET ligands are essential for the interpretation of clinical PET imaging results. The assurance of such quality control would enable comparisons of 6-[^{18}F]-DOPA/PET data obtained from various medical centers using different radiopharmaceutical procedures.

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Since 6-F-dopamine behaves like an adrenergic false neurotransmitter, the use of 6-[^{18}F]fluoro-L-dihydroxyphenylalanine (6-[^{18}F]-DOPA) in positron emission tomographic (PET) imaging of brain dopamine and the use of 6-[^{18}F]-catecholamines in imaging of autonomic tissues has been proposed (*1*). Garnett et al. (*2*) employed 6-[^{18}F]-DOPA instead of 5-[^{18}F]-DOPA (*3*) and obtained PET imaging of human brain dopamine neurons in vivo. The PET/6-[^{18}F]-DOPA imaging of brain dopamine neurons is the result mainly of a pre-synaptic synthesis and storage of the newly synthesized 6-F-dopamine (*4–7*), the decarboxylated metabolite of 6-F-DOPA in conjunction with a low rate of O-methylation of 6-F-DOPA, and 6-F-catecholamine by catechol-O-methyltransferase (*1,8–10*). Thus, PET/6-[^{18}F]-DOPA imaging of striatal dopamine disappeared completely in the MPTP-induced severely lesioned parkinsonian monkeys (*11,12*).

Several radiopharmaceutical methods for 6-[^{18}F]-DOPA preparation are employed in clinical PET studies of parkinsonism (*13–18*), and hence it is difficult to interrelate PET data reported from different medical centers with degree of dopamine depletion in the basal ganglia of study groups. According to most reports, radiopharmaceutical productions of 6-[^{18}F]-DOPA contain variable amounts of 2-[^{18}F]- and/or 5-[^{18}F]-DOPA (*13–17*). The brain uptake of 6-F-DOPA and the PET imaging of brain dopamine neurons have been shown to be completely abolished by pretreatment with eight neutral amino acids (*19*). It is generally believed that 2-F- and 5-F-DOPA may compete with the same amino acid transport mechanism through the blood-brain barrier as 6-F-DOPA. The presence of variable amounts of 2-F- and/or 5-F-DOPA in the 6-F-DOPA preparation may decrease imaging ratio of signal over background activity and compromise the dopamine imaging results by PET/6-[^{18}F]-DOPA procedures (*6,11,20*). Further-

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more, purified 2-[^{18}F]-DOPA produces no specific brain dopamine imaging in pre-clinical (*21*) and clinical (Firnau G: personal communication) PET studies because 2-F-DOPA is O-methylated extensively and is not a good substrate for L-DOPA decarboxylase in vivo. The use of highly purified ^{18}F-labeled 6-F-DOPA in future clinical studies is strongly indicated. The research goal of the current study was to establish quality control procedures for clinical use of 6-[^{18}F]-DOPA as a selective presynaptic PET imaging ligand for brain dopamine neurons.

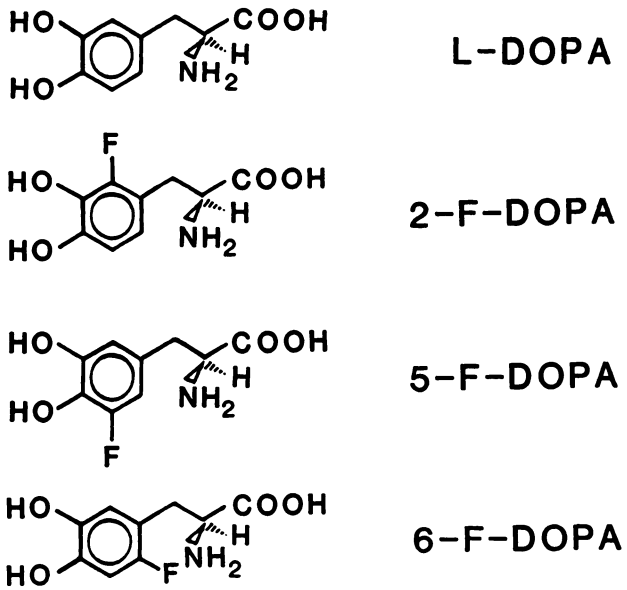

FIGURE 1
Chemical structure of L-DOPA and its catechol ring-fluorinated isomers.

MATERIALS AND METHODS

Synthesis of Fluoro-DOPA Standards and ^{18}F-Labeled 6-F-DOPA

Ring fluorinated DOPA derivatives, such as 2-F-, 5-F-, or 6-F-DOPA, were synthesized (*9,22,23,24*, Fig. 1) and used as standards in the present study. The radiopharmaceutical preparation procedures of ^{18}F-labeled 6-F-DOPA (*14–16*) were modified for routine on-site synthesis at the NIH cyclotron facility (Finn, et al: unpublished data). Stated briefly, ^{18}F-labeled fluorine gas was generated in a Japan Steel Works cyclotron (JSW Ltd., New York, NY) by the nuclear reaction of ^{20}Ne (d,α) ^{18}F and used to label acetylhypofluorite. Fluorine-18-labeled acetylhypofluorite was allowed to react with L-methyl-N-acetyl-[β-(3-methoxy-4-acetoxyphenyl)]alaninate (*16*). Radiofluorinated end products were hydrolyzed and purified by semipreparatory HPLC procedures in order to separate 6-[^{18}F]-DOPA from 2- or 5-[^{18}F]-DOPA and nonhydrolyzed intermediate precursors.

Quality Control Procedures for Clinical PET Ligand, 6-[^{18}F]-DOPA

Standardization of high performance liquid chromatographic procedures. The high performance liquid chromatographic (HPLC) procedures for fluorocatecholamines (*1*) were modified for the isolation of aromatic ring fluorinated L-DOPA isomers. The degassed ion-pairing mobile phase (per liter contains 0.1 g disodium salt of ethylenediamine tetraacetic acid (Na_2EDTA), 1.25 g heptanesulfonic acid, 5 ml triethylamine, 15 ml acetonitrile, and ~5 ml 85% phosphoric acid to adjust pH to 2.3), was delivered at 1.2 ml/min by a Waters model 6000A solvent pump (Millipore, Bedford, MA) to a C-18 reverse phase column (4.6 mm × 20 cm, 5μ, Altex). Catechol compounds were measured postcolumn by using an amperometric electrochemical detector (BAS, LC4B, West Lafayette, IN) with an oxidative potential of +0.725 V. The resolution and separation of the current HPLC procedure were examined by using a standard mixture containing 0.3 ng of DOPA, 2-F-DOPA, and 5-F-DOPA and 0.6 ng of 6-F-DOPA in 10 μl of 0.1N $HClO_4$. The sensitivity and linearity of the procedure were tested by using 0.025 ng to 80 ng of 6-F-DOPA standard.

Determination of chemical purity and specific activity of PET imaging ligand, 6-[^{18}F]-DOPA. Ten microliters of the purified radiopharmaceutical end-product of 6-[^{18}F]-DOPA (in saline for injection USP) were diluted 1:100 with 0.1N $HClO_4$. One hundred microliters of the diluent (1 μl of original parenteral solution of 6-[^{18}F]-DOPA) was injected onto the "standardized" HPLC system. The nonradioactive 6-F-DOPA in the injection solution was measured and quantified by the electrochemical detector. Every 15 sec the HPLC eluate was collected and assayed for ^{18}F radioactivity in a Beckmen model 8000 gamma counter (Fullerton, CA). The ^{18}F radioactivity was corrected for its half-life of 110 min to the time of administration of the PET imaging ligand to study subject. The relative retention time and oxidative potential of chromatographic peaks were compared with those of fluoro-DOPA standards. Two different HPLC columns and mobile phases were used to identify these fluorinated compounds.

The specific activity of the 6-[^{18}F]-DOPA was calculated from a formula which divides the total radioactivity of 6-[^{18}F]DOPA peak (nCi) by the amount of 6-F-DOPA peak (nmol) found in HPLC chromatograms. The reported specific activity of the imaging ligand was normalized to the time of administration or the beginning of PET scanning.

Stability of 6-[^{18}F]-DOPA and pyrogen test. Normally, this purified 6-[^{18}F]-DOPA was filtered through a 0.22-μm syringe filter unit (Millex-GS, Millipore, Bedford, MA) and intravenously administered (in 10 ml saline USP) to the study subject within 30 min after the end of radiopharmaceutical procedures. The remaining 6-F-DOPA injection solution (pH 4.5) turned a brownish color and formed a black precipitate within hours. Therefore, the current HPLC procedures were used further for an investigation of the stability of 6-F-DOPA solution (nonenzymatic oxidation and/or decayed products) with or without the addition of a preservative, 0.15% Na_2EDTA.

Apyrogenicity of the parenteral solution of 6-[^{18}F]-DOPA was determined by the limulus amebocyte lysate test (Food and Drug Administration Guideline HFN-320, 1988).

Statistical analysis. Results were reported as mean and 1

s.e.m. Student's t-test was used for statistical analysis with significant level set at $p < 0.05$.

RESULTS

The current HPLC procedures employed a 5-μ C-18 reverse phase column in conjunction with the ion-pairing mobile phase for the separation of L-DOPA and its catechol ring fluorinated derivatives. The relative retention times of L-DOPA, 2-F-DOPA, 5-F-DOPA, and 6-F-DOPA in one of the HPLC systems were 9.8, 11, 13, and 13.6 min, respectively (Fig. 2).

An amperometric electrochemical detector was used for a quantitative measurement of 6-F-DOPA. The oxidative potential of ^{18}F-labeled 6-F-DOPA sample was determined to be equivalent to the non-radioactive 6-F-DOPA standard which was different from that of 2-F-DOPA standard (Fig. 3). At the optimal oxidative potential of +0.725 V, the detection sensitivity for 6-F-DOPA was ~25 pg. A linear relationship of the injected amount of 6-F-DOPA standard to the nA output of the amperometric detector depicted as the peak height of the chart recorder was established. The linearity of the 6-F-DOPA standard curve extended from 0.025 to 80 ng in this current "standardized" HPLC procedure.

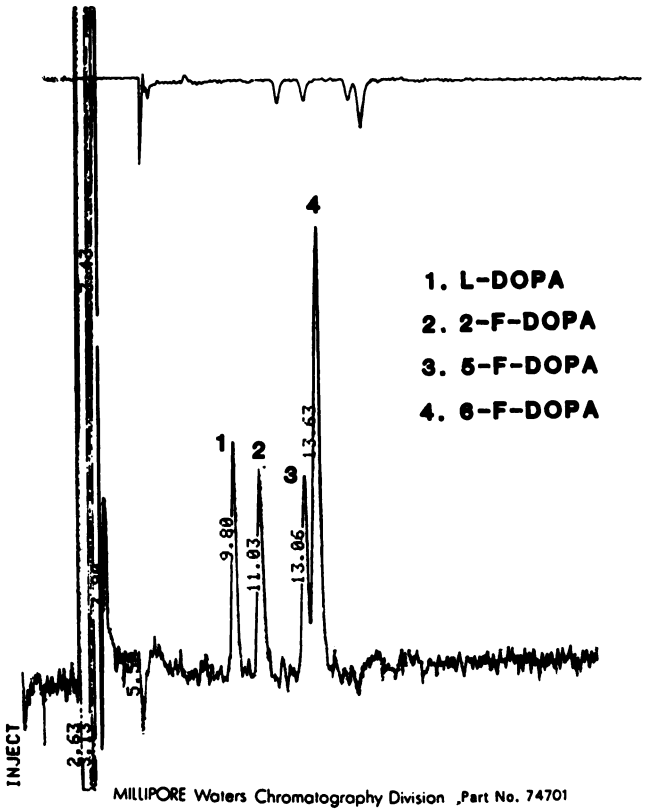

FIGURE 2
Separation and detection of L-DOPA, 2-F-DOPA, 5-F-DOPA, and 6-F-DOPA by HPLC-EC. A standard mixture of 0.6 ng of 6-F-DOPA and 0.3 ng of L-DOPA, 2-F-DOPA, and 5-F-DOPA was separated by using a 5-μ C-18 reverse phase column (4.6 mm × 20 cm, Altex) HPLC system. Each liter of ion-pairing mobile phase contained 0.1 g Na_2EDTA, 1.25 g heptanesulfonic acid, 5 ml of triethylamine, 15 ml acetonitrile, and ~5 ml of 85% phosphoric acid to adjust pH to 2.3. The L-DOPA and its ring-fluorinated structure isomers in the chromatogram were detected by using a BAS LC-4B amperometric electrochemical detector (EC, oxidative potential +0.725 V). The results were plotted by using a two-pen recording system (upper pen: 10 nA; lower pen: 1 nA full scale).

A typical HPLC chromatogram of the purified 6-[^{18}F]-DOPA (batch No. #0203) sample showed that this sample contained 98% 6-F-DOPA and 2% 2-F-DOPA (Fig. 4). Each μl of the 6-[^{18}F]-DOPA sample contained 163 ng (0.758 nmol) of nonradioactive 6-F-DOPA which was obtained either by peak height method or by digitalized peak area of the chromatogram. Furthermore, ^{18}F radioactivity in the HPLC eluate fractions indicated that two radioactive peaks were co-eluted with a similar retention time as those of 2-F- and 6-F-DOPA peaks, respectively (Fig. 4). The calculated radiochemical purity of the 6-[^{18}F]-DOPA sample was 97.1% which matched the chemical purity of 98% as stated above. Each μl of the 6-[^{18}F]-DOPA sample contained 350 nCi ^{18}F radioactivity at the beginning of PET imaging of brain dopamine neurons. Thus, the specific activity of the 6-[^{18}F]-DOPA sample (batch No. #0203) was estimated as 462 mCi/mmol (350 nCi/0.758 nmol). Quality control of eight consecutive batches of highly purified 6-[^{18}F]-DOPA sample used in a pre-clinical trial of PET imaging of brain dopamine neurons are summarized in Table 1. The chemical and radiochemical purity of the radiopharmaceutical product of 6-[^{18}F]-DOPA was greater than 97% with a total EOB activity of ~7 mCi per synthesis of this PET imaging ligand (specific activity: 365 ± 31 mCi/mmol; received parenteral doses 4.74 ± 0.13 mCi).

The relatively larger standard error for the specific activity of the 6-[^{18}F]-DOPA products was due to delays in the time of administration and/or the beginning of the actual PET imaging procedures. This delay varied from 15 min to 2 hr (within one half-life of ^{18}F isotope) during this pre-clinical trial of the PET/6-F-DOPA procedure. Repeated quality control studies of the same 6-[^{18}F]-DOPA sample in saline diluted solution indicated that the product is nonenzymatically oxidized and sensitive to light exposure and high temperature. The current HPLC procedure with electrochemical detection revealed at least two or/more oxidized metabolite peaks 2 hr after the end of synthesis (Fig. 5). 6-[^{18}F]-DOPA in a saline solution or 1% acetic acid solution was found to be sensitive to light which decreased by 20% after one hour exposure to light at room temperature. The addition of 0.15% Na_2EDTA to the 6-[^{18}F]-DOPA injection solution retarded the rate of this nonenzymatic oxidation (Fig. 6). A black precipitate was observed overnight in the 6-[^{18}F]-DOPA saline

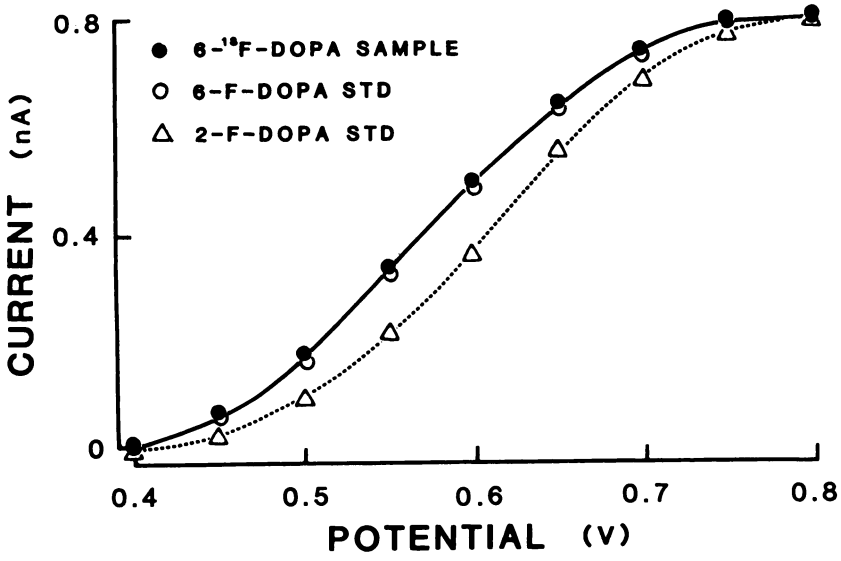

FIGURE 3
Determination of oxidative potential of 6-[^{18}F]-DOPA by amperometric electrochemical detection. The HPLC -EC procedure (see Method for detail) was used to compare the oxidative potential of 6-[^{18}F]-DOPA sample with the nonisotope 6-F-DOPA or 2-F-DOPA standard (0.5 ng each). At the optimal oxidative potential of +0.725 V, the BAS LC-4B detector yielded a high signal-to-noise ratio. The sensitivity and the linearity of the HPLC-EC procedure was measured by using 0.025 to 80 ng of 6-F-DOPA standards. The detection sensitivity was ~ 25 pg of 6-F-DOPA.

solution and indicated a formation of 6-F-dopa-chrome polymers which could not be measured by the current HPLC-electrochemical procedures.

The end-product of the parenteral solution of 6-[^{18}F]-DOPA was tested for endotoxin by the limulus amebocyte lysate test as recommended by the Food and Drug Administration (HFN-320). The test results revealed apyrogenicity nature of this PET imaging ligand. So far, none of the injected animals had developed positive pyrogenicity.

DISCUSSION

The current HPLC procedure using a 5-μ ultrasphere C-18 reverse phase column provided a complete sepa-

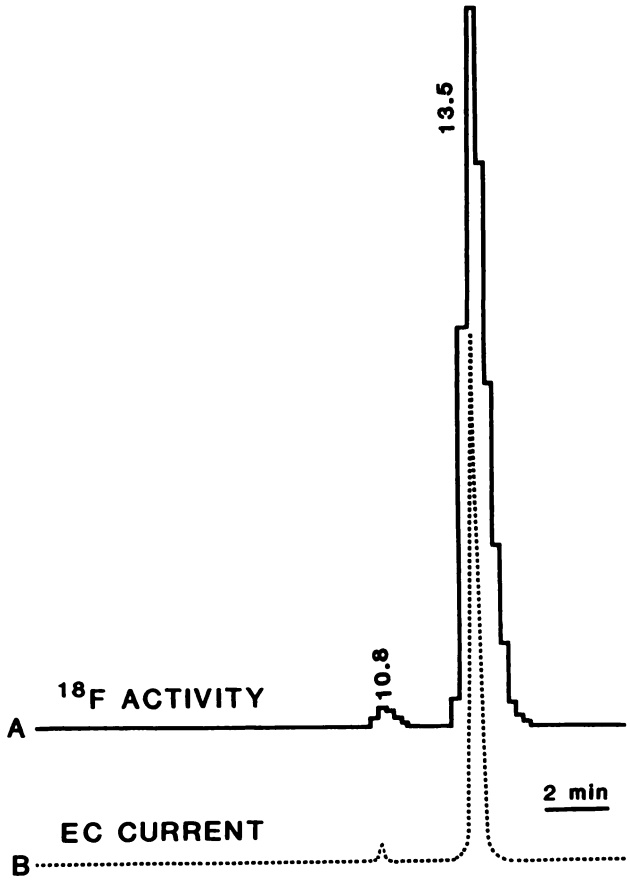

FIGURE 4
A typical HPLC chromatogram of 6-[^{18}F]-DOPA preparation. The radiochemical purity of 6-[^{18}F]-DOPA was measured by the ^{18}F radioactivity in the HPLC fractions (A) while the chemical purity of the PET ligand was determined by the amperometric detector (B). The HPLC elution pattern of the 6-[^{18}F]-DOPA sample was compared with external standards of L-DOPA, 2-F-, 5-F-, and 6-F-DOPA in the standardized HPLC system. The retention times of 2-F- and 6-F-DOPA standards in this HPLC run were 10.9 and 13.8 min, respectively. A decrease in the relative retention times for fluoro-DOPA standards was observed when this HPLC column was used repeatedly for more than 3 mo (see Table 1).

TABLE 1
Quality Control for 6-[^{18}F]-DOPA

Batch no.	HPLC retention time‡ (min)	Fluoro-DOPA	Chemical purity (%)	Radiochemical purity (%)	Specific activity* (mCi/mmol)	Dispensed doses* (mCi)
1. 0122	11.8	6-F-	97.6	96.7	231	5.2
	9.5	2-F-	2.4	3.3		
2. 0127	13.5	6-F-	98.1	97.3	313	4.2
	10.8	2-F-	1.9	2.7		
3. 0203	13.8	6-F-	98.1	97.1	462	5.1
	10.9	2-F-	1.9	2.9		
4. 0212	14.3	6-F-	96.0	96.6	267	4.4
	11.4	2-F-	2.5	2.7		
5. 0318	14.6	6-F-	97.7	97.5	407	2.45 × 2
	11.8	2-F-	1.8	2.5		
6. 0330	11.3	6-F-	95.8	93.7	420	4.3
	9.1	2-F-	4.2	6.3		
7. 0408	10.3	6-F-	95.6	95.1	467	4.8
	8.4	2-F-	4.4	4.9		
8. 0429	9.6	6-F-	97.6	98.0	355	2.5 × 2
	7.8	2-F-	2.4	2.0		
Mean ± s.e. (N = 8)	6-F-DOPA		97.1 ± 0.4	96.5 ± 0.5	365 ± 31	4.74 ± 0.13†
	2-F-DOPA		2.7 ± 0.4	3.4 ± 0.5		

* Specific activity was normalized to the time of administration of 6-[^{18}F]-DOPA at the beginning of PET imaging study.
† The total end-of-job yield for 6-[^{18}F]-DOPA is 6.4 ± 0.32 mCi.
‡ Decreased when the column was used for more than 2 mo.

ration of L-DOPA, 2-F-DOPA, 5-F-DOPA, 6-F-DOPA, and the oxidative metabolites of 6-F-DOPA. The sensitivity of the amperometric electrochemical detector (pg range) is at least 500-fold higher than that of uv detectors (ng range). Thus, the present quality control procedure requires only 5 to 30 μl of the end product of 6-[^{18}F]-DOPA which can be easily obtained from the tip of the injection syringe. The quality control data for 6-[^{18}F]-DOPA PET imaging ligand can be obtained within 20 minutes of the end of systhesis. Therefore, the radiochemical, chemical, and specific activity of 6-[^{18}F]-DOPA can be obtained and evaluated prior to the administration of this ligand to patients.

This quality control HPLC system standardized with proper external standards on each PET scan day, has been in use for more than one year. Due to its high sensitivity and resolution, this procedure can be used to identify not only contaminants, such as 2-F-DOPA and 5-F-DOPA, but also putative oxidized metabolites of 6-F-DOPA. In the early phase of the present preclinical PET project, this quality control procedure provided essential information for optimizing the synthetic and purification method to produce 6-[^{18}F]-DOPA. After using precautions to minimize the formation of 2-F- and 5-F-DOPA and a pre-column to remove the nonhydrolyzed intermediates in the synthesis of ^{18}F-labeled fluoro-DOPA, the routine production of 7 mCi of 6-[^{18}F]-DOPA PET imaging ligand of 97% purity was established at the NIH cyclotron facility (Finn, et al: unpublished data).

The stability of 6-[^{18}F]-DOPA preparation seems to depend upon several factors, but principally the nonenzymatic auto-oxidation of L-DOPA. Quality assurance to detect the presence of chemical impurities, such as 6-OH-DOPA, is imperative (Dr. D.J. Silvester: personal communication). The formation and/or accumulation of toxic amounts of 6-hydroxylated compounds is unlikely to occur in vivo because the specific activity of the 6-[^{18}F]-DOPA is below 0.5 Ci/mmol and only nonpharmacological dose of ^{18}F-labeled fluoro-DOPA (~5 mCi) is needed for PET imaging of brain dopamine neurons. The current HPLC procedure may be useful in investigating minute production of these putative neurotoxic substances in vitro since the amperometric electrochemical detector is sensitive to detect femtomoles of hydroxylated catechols, i.e., 6-hydroxy-L-DOPA (Chen et al: unpublished data). However, results of the current quality control procedure indicate that nonenzymatic auto-oxidation and polymerization plays a major role in causing destruction of the 6-F-DOPA molecule.

The addition of fluorine to the molecule of L-DOPA or catecholamines significantly alters its enzymatic reaction rate by catechol-O-methyltransferase and the

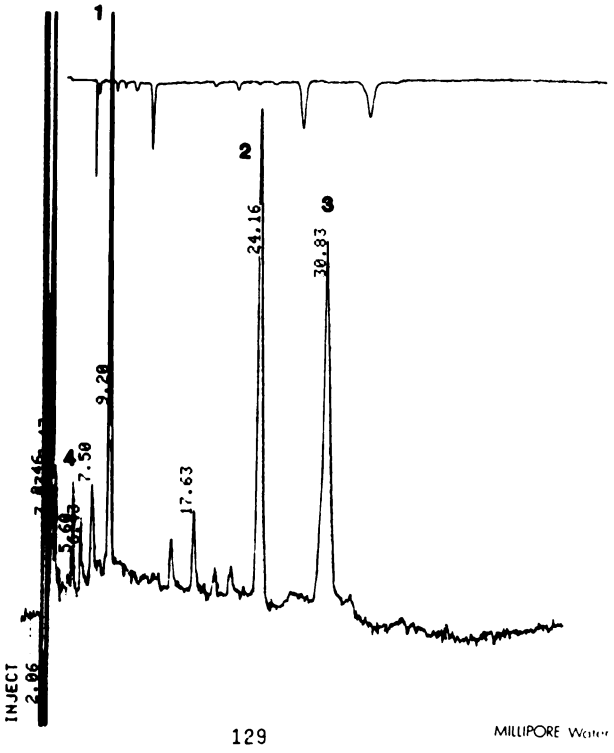

FIGURE 5
Separation and detection of nonenzymatic oxidative products of 6-[^{18}F]-DOPA by HPLC-EC procedure. The solution or color of 6-[^{18}F]-DOPA sample in saline for injection USP was found to darken with increasing time in the laboratory. Two hours after the end of synthesis, the HPLC-EC quality control of the 6-[^{18}F]-DOPA sample (1:100 dilution in saline) revealed several nonenzymatic oxidized products (peaks 2, 3, and 4) in addition to 6-F-DOPA (peak 1). The amperometric electrochemical detector cannot be used for the detection of polymerized 6-F-dopachrome (black precipitates).

affinity to sub-types of catecholamine receptors (*1,8,9, 24*). However, fluoro-DOPA appears to be as sensitive as L-DOPA to nonenzymatic auto-oxidation reactions caused by oxygen, light, temperature, and/or metal ions. The present HPLC system using an amperometric electrochemical detector detected two or more oxidative intermediates. This detector is not suitable for the detection of a completely oxidized and polymerized 6-F-dopachrome and/or dopachrome, seen as brownish black precipitates in the 6-F-DOPA preparation.

As shown in Figure 6, the addition of Na_2EDTA (0.15%) effectively retarded the auto-oxidation rate of 6-F-DOPA. The addition of Na_2EDTA as a preservative may be beneficial if the PET ligand is intended to be used 1 or 2 hr after the end of synthesis. Thus, it is recommended that the 6-[^{18}F]-DOPA end-product be stored in a cool and dark container for immediate use in PET scanning. The minimal specific activity of 6-[^{18}F]-DOPA needed to obtain PET imaging of brain dopamine neurons is estimated at ~50 mCi/mmol (Firnau G: personal communication). Since the calculated end-of-synthesis specific activity of the radiopharmaceutical synthesis of 6-[^{18}F]-DOPA was ~450 mCi/mmol, the current 6-[^{18}F]-DOPA product would allow a delay of PET scan up to 2 hr. Thus, one synthesis may support two consecutive PET scans on a typical day.

Fluorination of the catechol ring of L-DOPA occurs at carbon 2, 5, or 6 (*3,4,13–18*). 6-F-dopamine is taken up by and released from the dopamine neurons in the striatum and behaves like a false neurotransmitter (*1*). Furthermore, the 6-fluorinated L-DOPA or catecholamine is not O-methylated extensively as its 2- or 5-isomers (*1,7–10,20,24*). Since methylated DOPA accumulates relatively uniformly throughout the brain, the use of highly purified 6-[^{18}F]-DOPA, with reduced formation of methylated fluoro-DOPA could reduce the nonspecific background activity of PET/6-[^{18}F]-DOPA imaging of brain dopamine neurons. The major metabolite of 6-F-DOPA in the brain is identified as 6-F-dopamine rather than methylated 6-F-DOPA (*6,7, 10*). Thus, PET imaging of dopamine neurons in the basal ganglia was evident within 2 hr after administration of 6-[^{18}F]-DOPA but not after 2-[^{18}F]-DOPA (*21*, Firnau G: personal communication).

The current pre-clinical trial of the highly purified 6-[^{18}F]-DOPA (97% purity) produced a high PET dopamine neuron imaging ratio (signal over noise ranged from 2.4 to 3.5) in the caudate and putamen over that in the cortex or cerebellum (*25*). Since radiochemical purity of PET imaging ligands and its specific activity at the time of study are essential for interpretation of clinical PET imaging results. These results strongly indicate necessity to purify 6-[^{18}F]-DOPA prior to a systemic administration of this ligand in clinical PET studies. The assurance of this quality control procedure for 6-[^{18}F]-DOPA would enable medical centers to potentially compare 6-[^{18}F]-DOPA data. Despite differences in radiopharmaceutical methods (*13–18*), such PET studies of brain dopaminergic activity have already demonstrated their importance in visualizing the chemical brain damage of parkinsonism, but may also prove of utility in assessing the activity or turnover of dopamine in the living brain of patients with other neuropsychiatric disorders.

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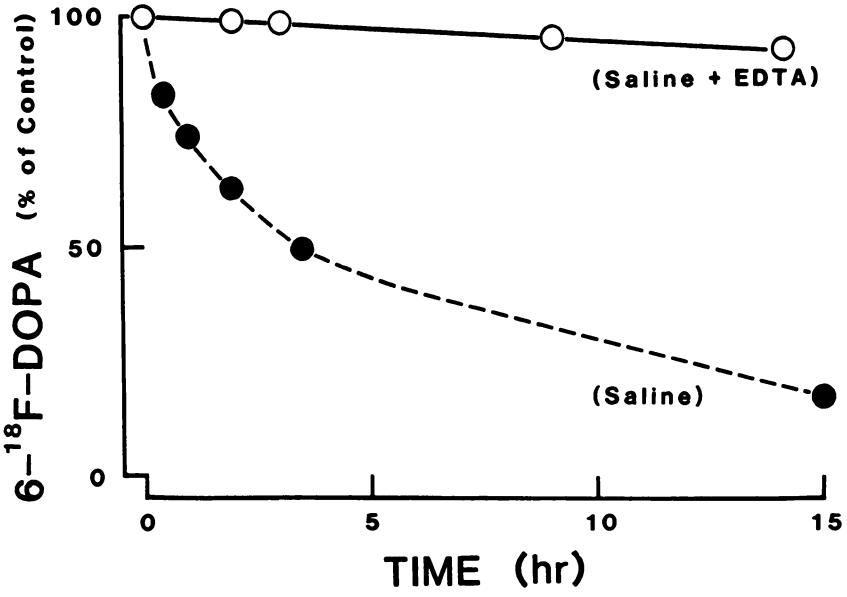

FIGURE 6
Prevention of auto-oxidation of 6-[^{18}F]-DOPA in saline for injection USP by Na_2EDTA. The stability of 6-[^{18}F]-DOPA in saline for injection USP was examined by using the HPLC-EC procedure. As shown in Figure 5, 6-F-DOPA was rapidly oxidized to form several products and it was found to be sensitive to high temperature and light. The addition of 0.15% Na_2EDTA to the 6-[^{18}F]-DOPA sample prevented this nonenzymatic auto-oxidation of 6-F-DOPA in parenteral saline solution.

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