ELSEVIER

Journal of Chromatography B, 693 (1997) 265–270

JOURNAL OF CHROMATOGRAPHY B

Determination of unchanged [^{18}F]dopamine in human and non-human primate plasma during positron emission tomography studies: a new solid-phase extraction method comparable to radio-thin-layer chromatography analysis

Rong Fu Wang[a,b,*], Christian Loc'h[b], Bernard Mazière[b]

[a]*Department of Nuclear Medicine, The First Hospital, Beijing Medical University, No.8, XiShiKu Street, Beijing 100034, China*
[b]*Service Hospitalier Frédéric Joliot, DRIPP-DSV-CEA, Place du Général Leclerc, Orsay 96401, France*

Received 1 October 1996; revised 24 January 1997; accepted 24 January 1997

Abstract

Routine determination of [^{18}F]DOPA and its metabolites in plasma is essential for assessment and quantification of presynaptic dopamine function in vivo using a modeling approach with positron emission tomography (PET). The determination of unchanged [^{18}F]DOPA from human and non-human primate plasma using solid-phase extraction (SPE) with Sep-Pak cartridges during PET dopaminergic studies is described here. The results from the studies showed that this new approach in comparsion to a method such as thin-layer chromatography (TLC) possessed a simplicity, rapidity and accuracy as well as good correlation between the two techniques ($p<0.0001$). A proposed procedure involving radio-analysis on alumina plates (Al_2O_3) was also developed with an excellent correlation compared to the conventional C_{18} plates ($r=0.96$). Thus it could be concluded that the SPE on either C_{18} or alumina cartridges (Waters) compared to radio-TLC analysis on C_{18} and alumina systems, appears to be a useful analytical method suitable for correcting the input arterial function in routine clinical PET neurotransmission studies.

Keywords: Dopamine

1. Introduction

6-[^{18}F]Fluoro-L-DOPA ([^{18}F]DOPA), a positron-emitting analog of L-DOPA, has been extensively used to evaluate the integrity of the central dopaminergic system in vivo using positron emission tomography (PET) [1–4]. Results from many studies indicated that [^{18}F]DOPA followed the metabolic pathway of L-DOPA both in the brain and in the periphery [5,6]. In the periphery, [^{18}F]DOPA is extensively metabolized to 6-[^{18}F]fluoro-3-O-methyl-L-DOPA (3-OMFD) by catechol-O-methyltransferase (COMT), and to [^{18}F]fluorodopamine by aromatic L-amino acid decardroxydase (AAAD); the free amine is rapidly sulphated by phenolsulfotransferase (PST) and remains as [^{18}F]fluorodopamine sulphate. In the striatum, [^{18}F]DOPA is readily decarboxylated to [^{18}F]fluorodopamine and low levels of [^{18}F]fluorodopamine metabolites such as 3,4-

*Corresponding author.

PII S0378-4347(97)00086-8

dihydroxy-6-[^{18}F]fluoprophenylacetic acid (FDOPAC) and 6-[^{18}F]fluorohomovanillic acid (FHVA) by monoamine oxidase (MAO) and COMT. However, for assessment and quantification of presynaptic dopamine function in vivo using a modeling approach with PET tracer an accurate knowledge of the input function of [^{18}F]DOPA is required, which is calculated from measurements of sequential unchanged radiotracer plasma concentrations. Previously reported techniques – reversed-phase high-performance liquid chromatography (HPLC) analysis [7,8] or alumina absorption [9] – have been used to determine and quantitate unchanged [^{18}F]DOPA and its metabolites in human and non-human primate plasma samples, but they proved expensive and time-consuming for clinical PET routine studies. Thus it is mandatory for routine clinical investigation with L-[^{18}F]fluoro-DOPA to design a more simple, convenient and reliable analytical technique. For this purpose, we propose here a new procedure involving Sep-Pak neutral alumina cartridges with comparison to TLC radio-analysis on C_{18} and alumina systems to measure the unchanged [^{18}F]DOPA during PET dopaminergic studies.

2. Experimental

2.1. Reagents

Methanol, sodium dihydrogen phosphate anhydrous (NaH_2PO_4), ethlendiaminetetra acetic (EDTA, $C_{10}H_{16}O_8N_2$), hydrochloric acid (HCl), 1-pentanesulfonic acid, sodium salt [$(CH_3(CH_2)_4SO_3Na$] and sodium metabisulfite ($Na_2S_2O_5$) were purchased from the Aldrich (Paris, France). All other chemicals were of reagent grade and used without further purification unless otherwise specified.

The pH 9.4 Tris–HCl (0.5 *M*) buffer was prepared by dissolving 3.5 g of Tris(hydroxymethyl)aminomethane ($C_4H_{11}NO_3$) in distilled water followed by distilling dropwise a certain volume of HCl, $10 \cdot 10^{-3}$ *M* EDTA and 0.1% $Na_2S_2O_5$.

The pH 3 of 0.1 *M* phosphate buffer was prepared by mixing 0.1 *M* NaH_2PO_4 to pH 3 with concentrated HCl, 0.1 m*M* EDTA and 2.6 n*M* 1-pentanesulfonic acid, sodium salt.

2.2. β^+-Emitting tracer: [^{18}F]DOPA

L-DOPA was purchased from commercial sources, and [^{18}F]fluoro emitting isotope was produced by cyclotron in our radiochemical laboratory. A 6-[^{18}F]DOPA preparation via electrophilic fluorination by the method of Adam et al. [10] with acetyl [18]hypofluorite was obtained in a radiochemical purity greater than 99% with a specific activity of 37–74 TBq (1–2 Ci)/mmol. The radioligand was routinely sterile and pyrogen-free.

2.3. Subjects of study

(1) Non-human primate study: twelve monkeys exposed to 1.5–12.5 mg/kg 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP) were deprived of food and liquids from midnight prior to PET study in Central Atomic Energy (CAE-Saclay, France); otherwise they were fed food and water ad libitum as per local animal science guidelines.

(2) Human study: eight healthy volunteers (five females, three males, mean age: 38 years) and eleven patients, eight with Parkinson's disease (PD) (six females, two males, mean age: 49 years) and three schizophrenics (two females, one male, mean age: 38 years) determined by physical examination, X-ray computed tomography (XCT) and nuclear magnetic resonance (NMR) were examined.

2.4. Tomographic imaging

PET imaging was performed on ECAT/B953 (Siemens, Hoffman Estates, IL, USA) with time of flight. Humans were positioned on supine with the imaging plane parallel to the orbito-meatal line, and their head placement was maintained by foam pillows and velcro strap restraints. The monkeys were anesthetized with 100–150 mg ketamine (i.m.) followed by intravenous injection (i.v.) of 25–50 mg of pentobarbital and their heads fixed in position with foam pillows. After injection of [^{18}F]DOPA, sequential emission scans were obtained beginning immediately after tracer administration using the following scanning sequence for monkeys: ten 90-s images, nine 5-min images, six 10-min images. The scanning sequence for human studies was: 30-s scan, four 3-min scans, five 10-min scans and three 20-min scans.

2.5. F-DOPA metabolites in plasma

After i.v. administration of 200 MBq of the radiotracer as a bolus injection via the venous catheter, rapid contralateral arterial blood samples were collected in heparinized tubes via the indwelling radial artery catheter in the human subjects and via the femoral artery catheter in monkeys. Whole blood samples (2 ml each for humans, 0.25 ml each for monkeys) were taken. Plasma metabolite determination was performed on samples drawn at 5, 10, 30, 45, 60 and 120-min after tracer injection. Samples were centrifuged, and the plasma separated.

For SPE measurements: 0.2 ml of plasma added with 0.5 ml of 0.5 *M* Tris–HCl buffer was poured on the C_{18} or neutral alumina cartridges prewetted with 5 ml of Tris–HCl buffer. The column was then washed with 2 ml of Tris–HCl buffer and 5 ml of distilled water to eliminate the radiolabelled metabolites. The remaining radioactivity on the column, corresponding to unchanged [^{18}F]DOPA, was measured in a gamma well counter (MR250, KONTRON), and compared with that of the untreated plasma sample.

For radio-TLC analysis: a 0.2 ml plasma sample, spiked with specimens of the main metabolites of F-DOPA, was deproteinized by addition of 2 ml of methanol and centrifuged. The methanolic supernatant was evaporated under reduced pressure and the residue, dissolved in 20 μl of methanol, was analyzed on C_{18} plates eluting with 0.1 *M*, pH 3 EDTA–phosphate buffer–methanol (95:5, v/v) or on neutral alumina plates eluting with the pH 9 of 0.5 *M* Tris–HCl buffer–methanol (50:50, v/v). The radioactivity distribution along the plates was then measured with a static radiochromatogram analyser and the control cold metabolites were revealed with iodide.

3. Results and discussion

3.1. Radio-TLC on C_{18} and alumina systems

Most interestingly, the results obtained with these original separation schemes in our work as compared to those of a previously reported solid-phase extraction technique from Chan et al. [11] and Wang et al. [12] are very comparable. The rate factors of [^{18}F]DOPA are respectively 0.85 and 0.00 with C_{18} and alumina systems. For every sample, the unchanged [^{18}F]DOPA, measured with the two proposed radio-TLC analytical procedures was quite similar and were highly correlated (r=0.998, $p<$ 0.0001) in comparison with results given by the control SPE approach.

With the C_{18} phase system, [^{18}F]DOPA (rate factor R_F=0.85) and the other four radioactive metabolites ([^{18}F]fluorodopamine R_F=0.75, 3-OMFD R_F=0.65, FDOPAC R_F=0.45 and FHVA R_F=0.21) can be identified in plasma samples (Fig. 1). However, a non negligible fraction of the radioactivity remains at the deposit point. This fraction which corresponds to radioactivity adsorbed on remaining proteins, should not be taken into account in the evaluation of the unchanged radiotracer fraction.

With the alumina system, [^{18}F]DOPA does not migrate, while 3-OMFD (R_F=0.75) is separated from FHVA (R_F=0.60) and from the other metabolites (R_F=0.40) (Fig. 2).

The data obtained for the determination of the unchanged fraction of 6-[^{18}F]fluoro-DOPA in human plasma of PET imaging using the two proposed analytical radio-TLC schemes and the control SPE are well correlated (Fig. 3), so radio-TLC using both RP-C_{18} and alumina plates would be a useful technique for analyzing [^{18}F]fluoro-DOPA and its radioactive metabolites in plasma samples for routine clinical PET studies [12]. Furthermore, a new radio-TLC analysis using alumina systems was developed and the data obtained are closely correlated with those of radio-TLC on C_{18} and of the control SPE.

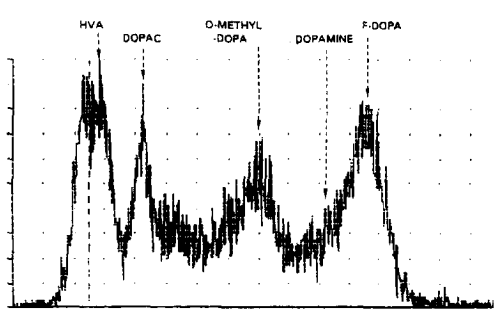

Fig. 1. [^{18}F]DOPA and its metabolites in human plasma of PET study measured by radio-TLC analysis on a C_{18} plate showed as follows: [^{18}F]DOPA R_F=0.85, [^{18}F]fluorodopamine R_F=0.75, 3-OMFD R_F=0.65, DOPAC R_F=0.45 and FHVA R_F=0.21.

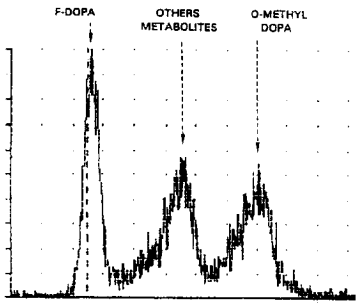

Fig. 2. [^{18}F]DOPA and its metabolites in human plasma of PET study measured by radio-TLC analysis on alumina plates exhibited [^{18}F]DOPA R_F=0.00, 3-OMFD R_F=0.75, FHVA R_F=0.60 and other metabolites R_t =0.40.

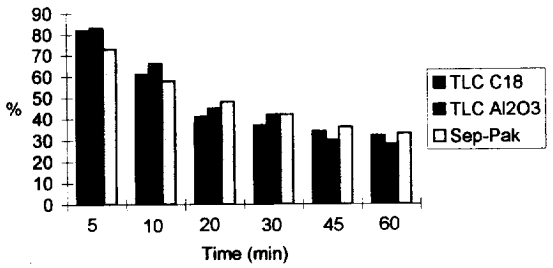

Fig. 3. Comparative characterization of unchanged [^{18}F]DOPA in human plasma measured by two radio-TLC analytical techniques with both C_{18} plates and alumina systems and by solid-phase extraction with Sep-Pak cartridges (Waters).

Table 1
The measured results of unchanged [^{18}F]DOPA in human and non-human primate plasma of PET study were obtained from SPE with Sep-Pak neutral alumina and radio-TLC analysis on an alumina system

Subjects	Time (min)	Sep-Pak cartridges		Coefficient of variation (%)
		[^{18}F]DOPA×100% total radioactivity (mean±S.D.)	TLC (alumina plate) (mean±S.D.)	
MPTP induced monkeys	5	80.7±4.5	82.2±10.4	0.9
(n=12)	10	60.9±9.2	60.0±12.3	1.5
	20	44.2±11.2	42.6±11.6	1.8
	30	32.8±10.5	30.9±9.1	2.9
	45	23.1±9.1	22.0±6.9	2.2
	60	20.4±7.7	19.8±4.4	1.5
Normal humans	5	69.5±5.1	69.0±0.8	0.3
(n=8)	10	55.5±6.0	53.2±1.5	7.2
	20	36.8±0.29	37.0±1.2	0.3
	30	28.0±1.8	30.0±0.6	3.4
	45	21.5±1.9	19.8±1.1	4.7
	60	19.8±1.2	18.9±2.9	4.5
Patients with PD	5	83.0±6.0	84.0±2.0	0.5
(n=8)	10	74.0±9.0	71.5±3.5	1.25
	20	61.5±9.5	60.5±5.5	0.5
	30	54.5±10.5	51.2±3.0	1.65
	45	51.0±8.0	48.5±2.5	1.25
	60	47.5±1.5	42.9±2.3	2.3
Schizophrenics	5	76.0±3.6	79.0±3.7	1.5
(n=3)	10	66.0±2.8	64.0±8.0	1.0
	20	40.3±6.2	43.6±1.8	1.6
	30	34.3±6.9	36.6±4.0	1.1
	45	31.0±6.0	29.0±0.8	1.0
	60	26.3±5.2	24.8±3.5	0.8

These two radio-TLC analytical methods, especially on alumina systems, economic and well correlated with control SPE, may be suitable for practical use. Moreover, SPE on Sep-Pak cartridges appears to be an easier and more useful technique which could replace HPLC or even radio-TLC for analyzing [^{18}F]fluoro-DOPA and its radioactive metabolites in human and non-human primate plasma samples for various PET neurotransmission studies [13,14].

3.2. SPE on neutral alumina cartridges

The results obtained in our study using SPE method with Sep-Pak cartridges for the measurement of [^{18}F]DOPA and its radioactive metabolites, parallel to those of a radio-TLC analytical method, are described in Table 1. This study exhibited an excellent correlation (r=0.988, p<0.0001) between the two proposed detection methods. The value of the coefficients of variation (mean±S.D.) data obtained with SPE and radio-TLC was of 2.6±1.3%. The slope was of 1.03 and the intercept was equal to 0.3%.

3.3. Comparison of both techniques: radio-TLC analysis and SPE measurements

For every plasma sample, the [^{18}F]DOPA and its metabolites, SPE using Sep-Pak neutral alumina cartridges and radio-TLC on alumina plates were performed in parallel. There was a good correlation (Figs. 4 and 5) between radio-TLC analysis on an alumina system versus SPE on a Sep-Pak Al_2O_3 [r=0.961, y=−5.2+(1.12x)], and between radio-

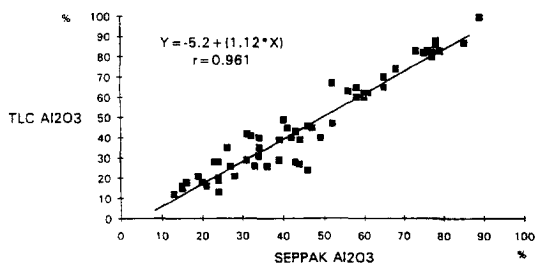

Fig. 4. Correlation of radio-TLC analysis on alumina system versus SPE on Sep-Pak Al_2O_3 cartridge for measurement and quantification of unchanged [^{18}F]DOPA in human and non-human primate plasma (mean±S.D., n=56).

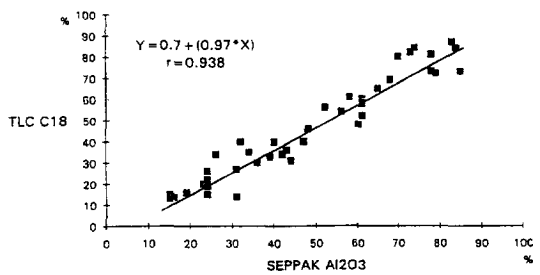

Fig. 5. Correlation of radio-TLC analysis on RP C_{18} versus SPE on Sep-Pak Al_2O_3 cartridge for measurement and quantification of unchanged [^{18}F]DOPA in human and non-human primate plasma (mean±S.D., n=56).

TLC RP-C_{18} plates versus SPE on Sep-Pak Al_2O_3 [r=0.938, y=0.7+(0.97x)].

4. Conclusions

We reported the results obtained from a new SPE technique with a simple, rapid, accurate and reliable Sep-Pak cartridge Al_2O_3 analytical method for assessment and quantification of unchanged [^{18}F]DOPA as well as its metabolites in human and non-human primate plasma during PET dopaminergic studies and compared them with those obtained from a classical radio-TLC analysis.

References

[1] E.S. Garnett, G. Firnau, C. Nahmias and R. Chirakal, Brain Res., 305 (1983) 169.
[2] E.S. Garnett, G. Firnau and C. Nahmias, Nature, 305 (1983) 137.
[3] H.N. Wagner, H.D. Burns, R.F. Dannals, D.F. Wong, B. Langstrom, T. Duelfer, J.J. Frost, H.T. Ravert, J.M. Link, S.B. Rosenbloom, S.E. Lukas, A.V. Kramer and M.J. Kuhar, Science, 221 (1983) 1264.
[4] W.P. Melega, S.T. Grafton, S.C. Huang, N. Satyamurtyz, M.E. Phelps and J.R. Barrio, J. Cereb. Blood Flow Metab., 11 (1991) 890.
[5] P. Comming, M. Hausser, W.P.W. Martin, J. Grierson, M. Adam, T.J. Ruth and E.G. McGeer, Biochem. Pharmacol., 37 (1988) 247.
[6] W.P. Melega, J.M. Hoffman, J.S. Schneider, C.H.K. Nissenson, M.M. Perlmutter, M.E. Phelps and J.R. Barrio, Soc. Neurosci., Abstr. Part 1, (1989) 107.
[7] G. Firnau, S. Sood, R. Chirakal, C. Nahmias and E.S. Garnett, J. Nucl. Med., 29 (1988) 363.

[8] W.P. Melega, A. Luxen, M. Permultter, C.H.K. Nissenson, M.E. Phelps and J.R. Barrio, Biochem. Pharmacol., 39 (1990) 1853.
[9] B. Boyes, P. Cumming, R. Martin and E.G. McGeer, Life Sci., 39 (1986) 2243.
[10] M.J. Adam, J.R. Grierson, T.J. Ruth and S. Jivan, Appl. Radiat. Isot., 37 (1986) 877.
[11] G.L.Y. Chan, K.A. Hewitt, B.D. Pate, P. Schofield, M.J. Adam and T.J. Ruth, Life Sci., 50 (1992) 309.
[12] R.F. Wang, C. Loc'h and B. Maziere, Br. J. Radiol., 65 (1992) 11.
[13] J. Delforge, J.A. Pappata, Y. Sanson, R.F. Wang, B. Bendriem, C. Crouzel and A. Syrota, J. Nucl. Med., 34 (Suppl.) (1994) 109.
[14] R.F. Wang, C. Loc'h, Y. Sanson, M. Varastet, M. Boettlander, Ph. Remy and B. Maziere, Neuroradiol., 34 (Suppl.) (1992) 61.