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Determination of the L-DOPA/L-tyrosine ratio in human plasma by high-performance liquid chromatography Usefulness as a marker in metastatic malignant melanoma

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Abstract

A first procedure was devised for determining 3,4-dihydroxyphenylalanine (L-DOPA) in human plasma by isocratic RP-HPLC coupled with electrochemical detection. A second procedure was devised for determining 3-hydroxyphenylalanine (L-tyrosine) in human plasma by isocratic RP-HPLC coupled with fluorescence detection. These methods were used to ascertain the L-DOPA/L-tyrosine ratio in plasma of patients with melanoma. Reference values were established by analysis of the L-DOPA/L-tyrosine ratio in the plasma of 35 normal healthy subjects. For 29 patients diagnosed as having melanoma without metastasis, the L-DOPA/L-tyrosine ($11.96\times10^{-5}\pm2.69\times10^{-5}$) level was not significantly different from that of 35 normal controls ($11.20\times10^{-5}\pm2.92\times10^{-5}$). However, this level was significantly increased ($p<0.05$) in the plasma of 17 patients with developing metastasis ($21.02\times10^{-5}\pm4.68\times10^{-5}$). © 1997 Elsevier Science B.V.

Keywords: 3,4-Dihydroxyphenylalanine; Tyrosine; Melanoma

1. Introduction

Cutaneous malignant melanoma has been one of the most rapidly increasing human malignant tumors over the last ten years. The incidence of malignant melanoma has risen dramatically in this century, particularly in Northern Europe, having doubled every 10 years in many countries [1]. The incidence in the United Kingdom and Germany is now roughly 10 per 100 000 per annum, giving an approximate lifetime risk of 1 in 200. Thus, the need for a specific, clinically reliable biochemical marker for early detection of metastasis has become increasingly urgent [2].

Melanoma, a malignant tumor arising from melanin-producing cells known as melanocytes, is characterized biochemically by abnormal tyrosine metabolism. Melanins are heterogeneous polyphenol-like biopolymers with a complex structure and a color varying from yellow to black. Two basic types exist in mammals: brownish-black eumelanins and yellow to reddish-brown pheomelanins. Both pigments are formed by a combination of enzymatically catalyzed and chemical reactions. The melanin biosynthetic pathway is critically regulated at the enzymatic level by tyrosinase (monophenol dihydroxyl phenylalanine:oxygen oxidoreductase EC 1.14.18.1.),

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a melanocyte-specific copper containing glycoprotein within specialized organelles called melanosomes [3,4]. Tyrosinase is a key factor in melanogenesis since it catalyzes the rate-limiting step in melanin biosynthesis, i.e. hydroxylation of 3-hydroxyphenylalanine (L-tyrosine) to 3,4-dihydroxyphenylalanine (L-DOPA), and subsequent oxidation to DOPAquinone [5]. DOPAquinone is oxidized to DOPAchrome, which gives dihydroxyindoles (5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid). These dihydroxyindoles are further oxidized to produce eumelanin (brown/black pigment). Conversely, if DOPAquinone encounters cysteine or glutathione, a pheomelanin (yellow/red pigment) is formed via cysteinyldopas, 5-S-cysteinyl-L-DOPA being the major metabolite [6–8] (Fig. 1).

As the biochemistry of malignant melanoma is characterized essentially by accelerated production of melanin pigment, many reports have suggested that the quantification of melanin precursors/metabolites in urine and plasma (serum) [9–11], or measurements of tyrosinase activity [12], might facilitate detection of melanoma metastases. As L-tyrosine serves as the starting material for the biosynthesis of melanin, the main objective of this paper was to further investigate the role of plasma L-DOPA/L-tyrosine level, the key intermediary at the initial step of melanogenis, as a possible specific biochemical marker for malignant melanoma. Accordingly, we determined the concentration of L-DOPA and L-tyrosine in plasma samples from 46 patients with biopsy-proven melanoma and 35 healthy subjects. We also evaluated L-DOPA and L-tyrosine concentrations in a subgroup which developed metastases.

For identification and quantification of trace amines such as norepinephrine (NE), epinephrine (E) and dopamine (DA) in biological samples, ion-pair RP-HPLC with coulometric detection (CD) has

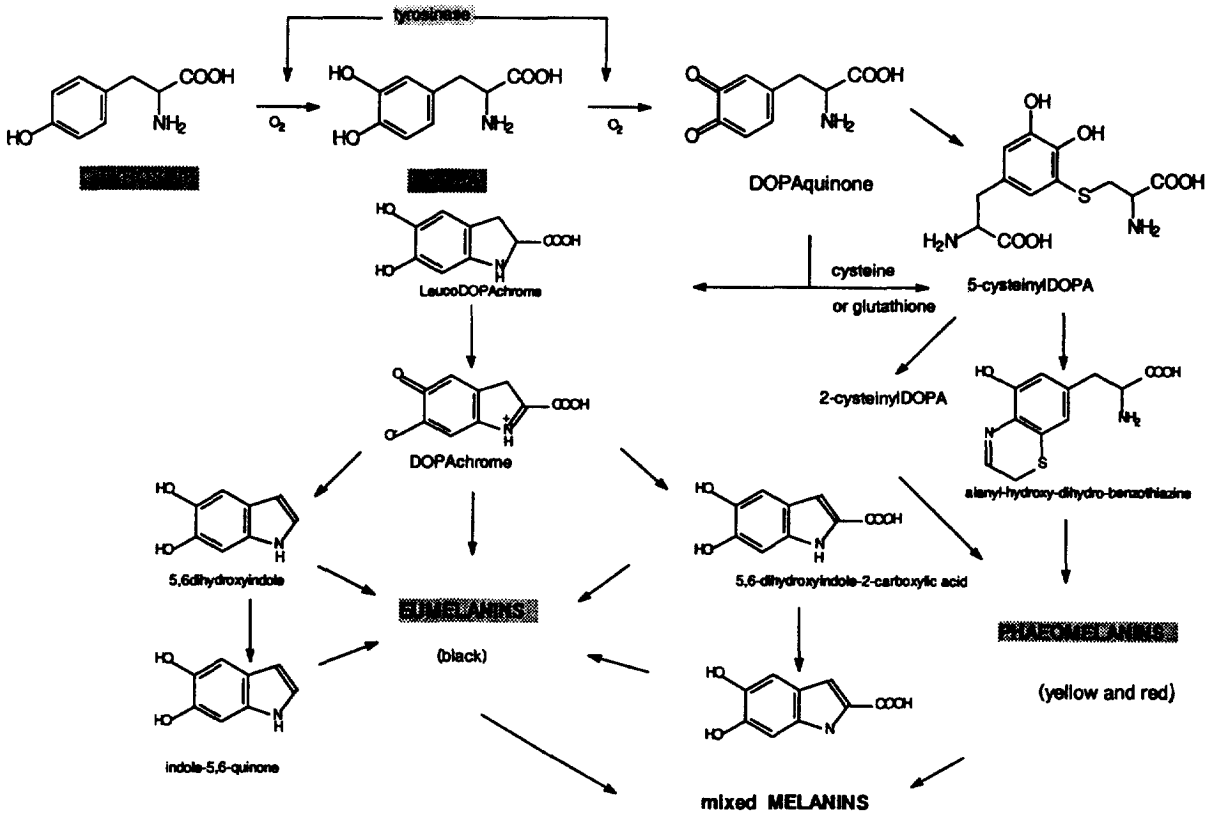

Fig. 1. Melanin biosynthesis.

become the method of choice because of its high sensitivity and specificity [13–16]. The ability to evaluate L-dihydroxyphenylalanine (L-DOPA) and 3,4-dihydroxyphenylacetic (DOPAC) additionally [17–19] makes HPLC with CD an attractive technique for a wide range of applications. This paper shows that minimal changes in experimental conditions may disturb the quality of the analytical process. Thus, a method was developed for simultaneous determination of NE, E, DA, L-DOPA and DOPAC from plasma using adsorption on alumina and HPLC with CD. This method was then applied to the quantification of L-DOPA in human plasma.

Several analytical methods for detection and quantification of tyrosine in plasma have been reported [20–22]. The present study describes the determination of L-tyrosine in plasma by a highly sensitive and selective method using HPLC with fluorimetric detection.

2. Experimental

2.1. Chemicals and reagents

Epinephrine hydrochloride, norepinephrine hydrochloride, L-tyrosine, L-dihydroxyphenylalanine, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxybenzylamine (DHBA), *p*-hydroxyphenylacetic acid (PHPA), octanesulfonic acid sodium salt monohydrate and sodium laurylsulfate were obtained from Sigma (Munich, Germany). Methanol (HPLC grade) and all other chemicals (analytical grade) were obtained from Merck (Darmstadt, Germany). Alumina (activity grade I) for chromatography was purchased from Merck and prepared by the method of Anton and Sayre [23]. Milli Q water (Millipore, Saint Quentin/Yvelines, France) was used.

2.2. Preparation of stock solutions and spiked standards

Stock solutions of L-DOPA, L-tyrosine, DHBA (I.S.) and PHPA (I.S.) were prepared in 0.01 *M* hydrochloric acid at concentrations of 5 m*M*, 1 m*M*, 0.1 m*M* and 0.25 m*M*, respectively. These solutions were stored for three months at −20°C. Working standard solutions prepared each day by dilutions with Milli Q water were used to spike the plasma samples prior to extraction.

Pooled plasma samples from healthy volunteers were used for validation of the method.

2.3. Apparatus

The chromatographic apparatus was equipped with a 510 HPLC pump (Waters, Milford, MA, USA) and a WISP 710 B automatic sample injector (Waters). Separations were performed on a C_{18} reversed-phase analytical column (125×4 mm I.D.) with an integrated precolumn (4×4 mm), both filled with Nucleosil (5 μm particle size) supplied by Merck. Temperature was kept stable at 25°C.

To quantify L-DOPA, the system was connected to a coulometric electrochemical detector (Model 5100A, ESA, Bedford, MA, USA) equipped with an analytical cell (Model 5011, ESA). The electrochemical cell was operated in oxidative mode, with the detector potential set at +0.35 V.

To quantify L-tyrosine, the system was connected to a fluorimetric detector (Model RF 535, Shimadzu, Kyoto, Japan). Fluorescence was monitored with excitation at 275 nm and emission at 305 nm. All separations were carried out at room temperature.

2.4. Mobile phase

To quantify L-DOPA, the mobile phase was composed of 0.05 *M* phosphate buffer, sodium octanesulfonic acid (concentrations ranging from 0.80–2.50 m*M*) and 0.020 *M* sodium ethylenediaminetetracetic acid (methanol volumic fraction: 3%). The pH was adjusted to between 2.6 and 3.0 with 1 *M* phosphoric acid. Isocratic elution was performed at a flow-rate of 1 ml min^{-1}.

To quantify L-tyrosine, the mobile phase was composed of 0.08 *M* acetate buffer, 0.020 *M* sodium ethylenediaminetetracetic acid and 1.80 m*M* sodium laurylsulfate (methanol volumic fraction: 10%). The pH was adjusted to 3.8 with 1 *M* acetic acid. Isocratic elution was performed at a flow-rate of 1 ml min^{-1}.

Prior to use, the eluent was filtered through a 0.2-μm membrane filter (Millipore) and sonicated for 10 min.

2.5. Sample preparation

Blood samples were drawn by venous puncture in 7-ml glass Hemoguard lithium heparin (LH) Vacutainer tubes (Becton Dickinson, Meylan, France). Samples were centrifuged at 4°C, and the plasma was then removed and stored at −20°C until analysis.

For L-DOPA determination, plasma samples (1 ml) were spiked with the internal standard, 3,4-dihydroxybenzamide (50 μl, 200 n*M*), in 17×110 mm conical polypropylene tubes; and L-DOPA, DOPAC and catecholamines were isolated by an alumina adsorption procedure [23]. After addition to 50 mg alumina of 0.4 ml of 3 *M* Tris buffer (pH 8.6) and 0.100 ml of 10% EDTA, the test tubes were vortex-mixed for 5 min. The alumina was centrifuged for 10 min at 4°C (at 2000 *g*), and the supernatant was discarded. The alumina pellet was washed 3 times for 10 min in 5 ml of distilled water, with centrifugation each time and discarding of the supernatant. To the washed alumina, 800 μl of 0.2 *M* $HCLO_4$ were added, followed by vortexing for 10 min. After centrifugation, the supernatant was transferred into a clean injector vial. A 180-μl volume of eluate was injected directly into the column. Alternatively, samples were kept in a refrigerator until just before injection. As soda-lime glass tubes proved unsuitable even for short-term storage of acidified mixtures containing L-DOPA and DOPAC, only polypropylene tubes were used [24].

For L-tyrosine determination, the plasma sample (1 ml) spiked with the internal standard, 50 μl of 2.5 m*M* *p*-hydroxyphenylacetic acid, was deproteinized by addition of 0.5 ml of 1 *M* trichloroacetic acid. The mixture was centrifuged for 10 min at 4°C (at 2000 *g*). Ten microliters of supernatant were then injected directly into the liquid chromatograph.

2.6. Recovery

Extraction recoveries were determining by comparing peak heights of plasma spiked with known amounts of drug (corrected for endogenous levels) processed according to the described method versus peak heights of the same concentrations prepared in purified water injected directly onto the analytical column. Each sample was determined in five replicates.

Extraction efficiencies were also determined for internal standards.

2.7. Precision

Precision concerned repeatability and reproducibility, which were measured for the entire procedure (extraction and HPLC). The intra-day repeatability of methods was evaluated by same-day analysis of six replicates of spiked samples at each of three concentrations. Inter-day reproducibility was assessed by performing analyses of spiked samples at three concentrations. The procedure was repeated on different days ($n=6$). Precision was expressed as a coefficient of variation (C.V.) by calculating the standard deviation as a percentage of the mean concentration found.

2.8. Statistical analysis

Results are shown as mean S.D. ($\bar{X}\pm\sigma$). Comparisons of means were evaluated with Student's *t*-test. Values of $p<0.05$ were considered statistically significant.

3. Results and discussion

3.1. HPLC analysis

3.1.1. Mobile phase

To quantify L-DOPA, a systematic study was required to establish the optimum composition of the mobile phase. The compounds to be separated had different polarities, namely basic (NE, E, DA, DHBA: internal standard) and acidic (DOPAC, L-DOPA), but were extractable in the same way.

Minimal changes in the composition of the mobile phase markedly interfered with the separation of these compounds. An increase in methanol concentration led to a decrease in k' (capacity factor) values for all compounds without affecting the order of elution. Optimal resolution was achieved at a methanol volumic fraction of 3%.

Minimal changes in acid concentrations in the eluent interfered with the retention of amines without

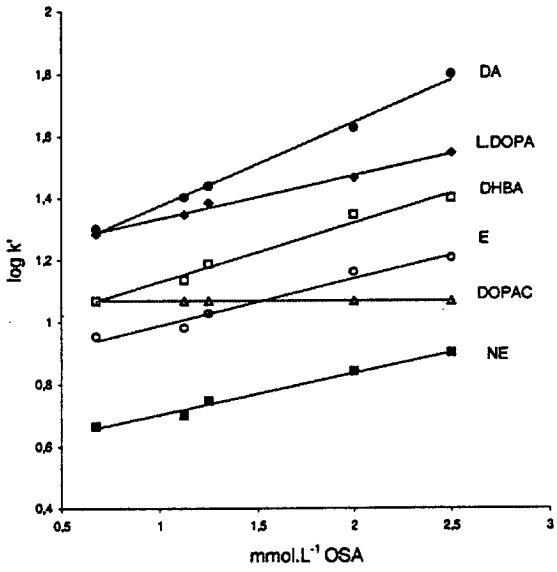

Fig. 2. Variation of the capacity factor (k') logarithm of (■) NE, (○) E, (●) DA, (♦) L-DOPA, (△) DOPAC and (□) DHBA (I.S.) as a function of counter-ion (OSA) concentration in mobile phase.

altering that of DOPAC; 1.125 m*M* of ion-pair additive was found to be optimal (Fig. 2).

Minor pH fluctuations did not affect the retention of DOPAC and catecholamines, whereas a modification in pH led to changes in L-DOPA retention. As pH increased, the elution time of L-DOPA decreased. Changes in pH of less than one-tenth of a unit could produce shifts in L-DOPA retention time of as much as 1 min (Fig. 3). Separation was obtained at pH 2.80. At any other pH, there was a risk of coelution of L-DOPA with DOPAC, DHBA (internal standard) or catecholamines.

The optimal mobile phase consisted of 0.050 *M* phosphate buffer, pH 2.8 (containing 1.125 m*M* sodium octanesulfonic acid and 0.020 *M* sodium ethylenediaminetetracetic acid), with a 3% methanolic fraction.

For L-tyrosine quantification, the selection of the internal standard PHPA was based on the fact that it was spectrofluorometrically active, did not interfere with analysis of the analyte and was suitable for the proposed extraction.

Methanol content, pH values and sodium laurylsulfate concentration influenced the capacity factor k' of the compounds. A higher percentage of methanol reduced the capacity factors for all compounds; sodium laurylsulfate concentration influenced the retention for L-tyrosine; and a low pH increased the retention time for PHPA (internal standard). When 0.08 *M* acetate buffer, pH 3.8

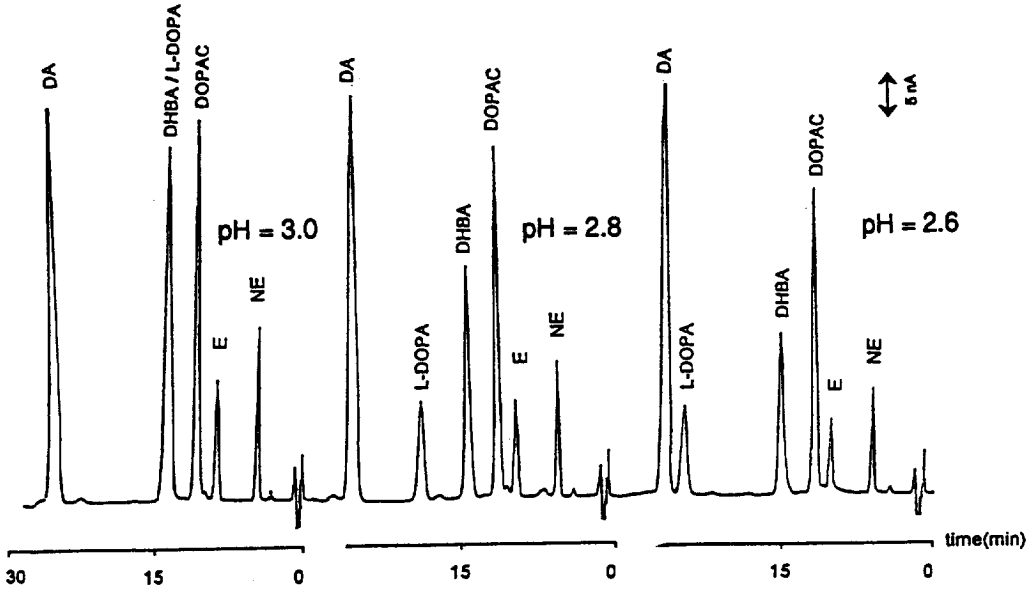

Fig. 3. Variation of retention time of NE, E, DA, L-DOPA, DOPAC and DHBA (I.S.) as a function of pH in mobile phase. As the pH of the mobile phase increases, the retention time of L-DOPA decreases.

[containing 1.8 m*M* sodium laurylsulfate and 0.020 *M* EDTA)–MeOH (90:10, v/v)], was used, all compounds tested were properly separated within 10 min.

3.1.2. Chromatograms

Representative chromatograms of a healthy human plasma sample (A) and a spiked plasma sample (B) are shown in Fig. 4 for L-DOPA determination and in Fig. 5 for L-tyrosine determination. Extraction conditions are described in Section 2.5.

3.1.3. Selectivity

HPLC coupled with electrochemical detection has greatly reduced drug-induced interference [25]. Fig. 4 shows chromatograms of a plasma sample (A) and the same plasma spiked with NE, E, DOPAC, L-DOPA and DA (B). None of the endogenous compounds in plasma, extractable in the same way, interfered with the retention time of L-DOPA or the internal standard. Fig. 5 shows chromatograms of a plasma sample (A) and the same plasma sample spiked with L-tyrosine and PHE (B). PHE did not interfere with the retention time of L-tyrosine or the internal standard.

The drugs or metabolites examined, none of which interfered with the assay, included acetaminophen, acetylsalicylic acid, labetalol, dihydroxycaffeic acid, α-methylDOPA and dihydroxyphenylglycol.

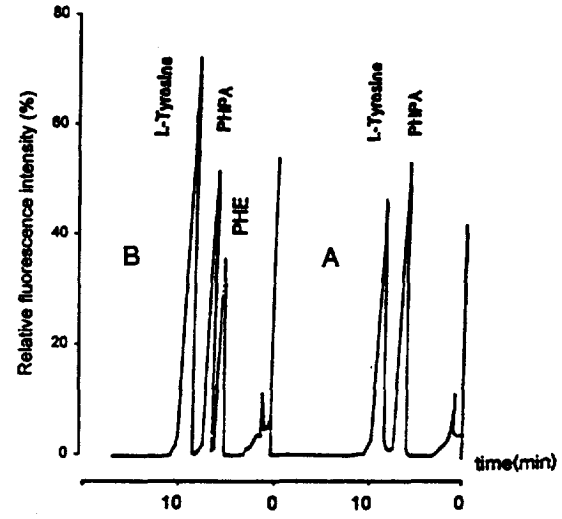

Fig. 5. Chromatograms obtained from analysis of (A) a healthy human plasma containing L-tyrosine (85.5 μ*M*) and (B) the same human plasma spiked with 50 nmol of L-tyrosine and PHE (phenylalanine). Chromatographic conditions: mobile phase; 0.08 *M* acetate buffer, pH 3.8 (containing 1.8 m*M* sodium laurylsulfate and 0.02 *M* EDTA)–MeOH (90:10, v/v); flow-rate 1 ml min^{-1}; column, 125×4 mm I.D., RP-18 coupled with an in-line guard column. Fluorescence detection wavelengths were set at 275 nm (excitation) and 315 nm (emission).

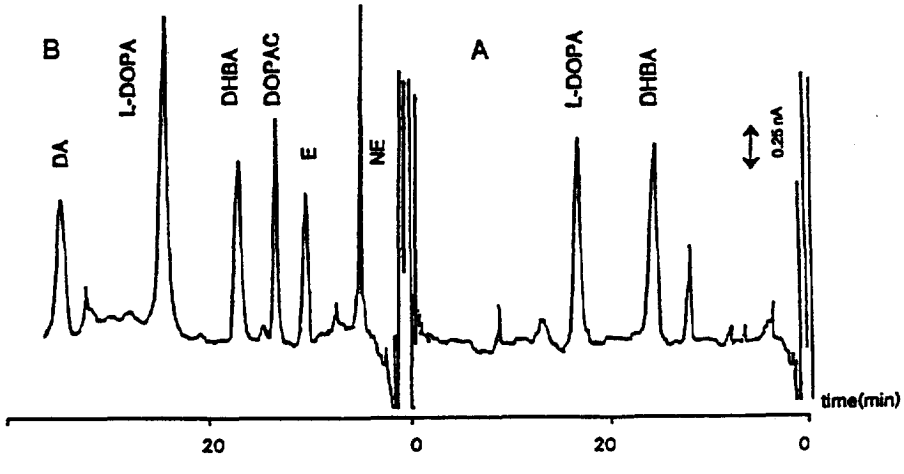

Fig. 4. Chromatograms obtained from analysis of (A) a healthy human plasma containing L-DOPA (7.9 n*M*) and (B) the same human plasma spiked with 5 pmol of NE, E, DOPAC and L-DOPA and 10 pmol of DA. Chromatographic conditions: mobile phase; 0.05 *M* phosphate buffer, pH 2.8 (containing 1.125 m*M* sodium octanesulfonic acid and 0.02 *M* EDTA)–MeOH (97:3, v/v); flow-rate 1 ml min^{-1}; column, 125×4 mm I.D., RP-18 coupled with an in-line guard column. Coulometric detection was set at +0.35 V.

3.1.4. Recovery

Recoveries of L-DOPA and especially DOPAC were typically lower than those of catecholamines and dihydroxybenzylamine (DHBA) used as internal standard [24]. As the poor accuracy and reliability of plasma DOPAC determination limited the use of this metabolite as a marker, we tested several modifications intended to improve the analytical recovery and reproducibility of assays for simultaneous measurement of plasma levels of L-DOPA, DOPAC and other endogenous catechols. For maximum sensitivity, it is essential to desorb in a minimum volume of acid compatible with maximum recovery. The volume and properties of the eluent were studied as possible influences on the elution procedure. Maximum analytical recoveries were obtained with 800 μl of 0.2 *M* perchloric acid.

Mean recovery of catecholamines averaged 60.5±4.3% and was not statistically different over the range of concentrations studied. The average extraction recoveries of L-DOPA and DOPAC at the low (3 n*M*) concentration were 72.3±2.5% and 46.9±3.2% respectively, and were 74.8±2.3% and 48.8±2.8% at the higher (50 n*M*) concentration. Mean recovery of the internal standard (DHBA) averaged 71.3±1.2% at a concentration of 9.5 n*M*.

The average extraction recovery of L-tyrosine was 87.9±1.9% at the low (25 μ*M*) concentration and 88.2±1.2% at the higher (200 μ*M*) concentration. Mean recovery of the internal standard (PHPA) averaged 92.1±2.3% at a concentration of 120 μ*M*.

3.1.5. Electrochemical characteristics

Voltammograms for oxidation of catecholamines, L-DOPA and DOPAC have been described in the literature [26,27]. These compounds are easily oxidizable between +0.15 and +0.20 V. The optimal oxidation potential was found to be +0.35 V.

3.1.6. Linearity and detection limits

Linearity was examined by analyzing six spiked plasma concentrations (L-DOPA: 1, 5, 10, 20, 50 and 100 n*M*, L-tyrosine: 10, 20, 50, 100, 200 and 500 μ*M*) on four different days. For each curve, the peak height ratio of drug to internal standard was calculated and plotted against the respective concentration of L-DOPA or L-tyrosine. Linear regression analysis was performed to calculate the slope, intercept and correlation coefficient of the calibration curve. Calibration parameters obtained with four different curves are reported in Table 1. Good linear responses, expressed in terms of the regression coefficient (*r*) and slope reproducibility, were observed for each compound.

Table 1
Reproducibility of four calibration curves

Compound	Slope (*m*)	Intercept (*b*)	*r*
L-DOPA	0.141	0.734	0.9992
	0.138	0.794	0.9995
	0.129	0.861	0.9991
	0.135	0.822	0.999
Mean	0.136	0.803	0.9992
S.D.	0.0051	0.0538	0.0002
C.V.(%)	3.8		0.02
L-Tyrosine	0.0103	0.4556	0.9998
	0.01	0.478	0.997
	0.0101	0.4886	0.9993
	0.0107	0.4564	0.9998
Mean	0.0103	0.470	0.9990
S.D.	0.0003	0.0164	0.0013
C.V. (%)	3.0		0.13

For a signal-to-noise ratio of at least three, assay detection limits corresponded to a plasma concentration of 0.25 n*M* for L-DOPA and 2.5 μ*M* for L-tyrosine.

3.1.7. Precision

The intra-assay and inter-assay precision for L-DOPA and L-tyrosine are shown in Table 2. Precision was evaluated by analyzing six replicates of three different samples containing L-DOPA (2.5 n*M*, 40

Table 2
Intra-day ($n=6$) and inter-day ($n=6$) assay

Concentration added	C.V. (%)	
	Intra-assay	Inter-assay
L-DOPA (nM)		
2.5	2.9	3.9
40	2.4	3.4
80	1.9	3.0
L-Tyrosine (μM)		
25	2.4	4.5
150	2.2	4.3
400	1.6	3.9

Table 3
Plasma levels (mean±S.D.) of L-DOPA, L-tyrosine and L-DOPA/L-tyrosine

	N	M^-	M^+
L-DOPA (n*M*)	6.48±1.45	6.85±1.55	10.05±2.65
L-Tyrosine (μ*M*)	60±14	59±16	48±10
L-DOPA/L-tyrosine ($\times 10^5$)	11.20±2.92	11.96±2.69	21.02±4.68

Samples from healthy subjects (N) (n=35), metastasis-free melanoma patients (M^-) (n=29) and patients with metastatic melanoma (M^+) (n=17).

n*M*, 80 n*M*) or L-tyrosine (25 μ*M*, 150 μ*M*, 400 μ*M*). Precision, expressed as C.V. (%), was less than 5%.

3.2. Preliminary patient study

Plasma samples from 35 healthy subjects and 46 melanoma patients were analyzed. The results for determinations of L-DOPA and L-tyrosine concentrations and the L-DOPA/L-tyrosine ratio are shown in Table 3 and Fig. 6. There were no significant differences in L-DOPA and L-tyrosine levels or the L-DOPA/L-tyrosine ratio between the group of healthy subjects (N) and patients with metastasis-free melanoma (M^-) (n=29). However, the increase in L-DOPA concentration and the decrease in L-tyrosine concentration were significant between the group of healthy subjects and patients with metastatic melanoma (M^+) (n=17), probably reflecting the increased activity of tyrosinase. The L-DOPA/L-tyrosine ratio (N: $11.20\times10^{-5}\pm2.92\times10^{-5}$) became significantly higher (M^+: $21.02\times10^{-5}\pm4.68\times10^{-5}$) ($p<0.05$) when metastases were associated.

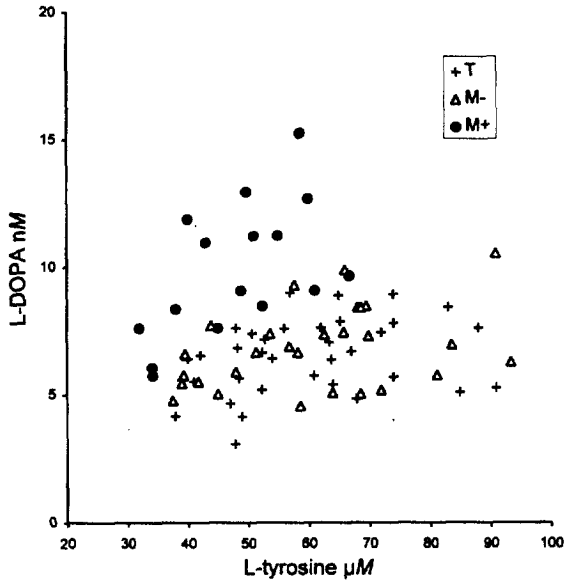

Fig. 6. Plasma level data for L-tyrosine (reported on the x-axis) and L-DOPA (reported on the y-axis) from healthy subjects (N), metastasis-free melanoma patients (M^-) and patients with metastatic melanoma (M^+). The L-DOPA/L-tyrosine ratio for all data were obtained by y/x.

4. Conclusion

The results of this study indicate the importance of the plasma level of L-DOPA/L-tyrosine in estimations of melanoma metastasis. However, the data reported here are preliminary and need to be confirmed by a study based on large-scale samples. Further studies are required to evaluate the clinical significance of the L-DOPA/L-tyrosine level in human plasma as a marker indicative of (1) the progression of disease and metastasis development, (2) the efficacy of therapy and (3) the prognosis. This method will probably prove effective in the follow-up of patients with melanoma.

References

[1] P. Boyle, P. Maisonneuve, J.-F. Dore, Br. Med. Bull. 51 (1995) 523.
[2] T. Horikoshi, S. Ito, K. Wakamutsu, H. Onodera, H. Eguchi, Cancer 73 (1994) 629.
[3] C. Jimenez-Cervantes, J.C. Garcia-Borron, P. Valverde, F. Solano, J. Lozano, Eur. J. Biochem. 217 (1993) 549.
[4] C. Jimenez-Cervantes, F. Solano, T. Kobayashi, K. Urabe, V. Hearing, J. Lozano, J.C. Garcia-Borron, J. Biol. Chem. 269 (1994) 17993.
[5] A. Sancez-Ferrer, J.N. Rodriguez-Lopez, F. Garcia-Canovas, F. Garcia-Carmona, Bioch. Biophy. Acta 1247 (1995) 1.

[6] N. Smit, J.M. Latter, S. Naish-Byfield, W. Westerhof, S. Pavel, P. Riley, Biochem. Pharm. 48 (1994) 743.
[7] P. Aroca, K. Urabe, T. Kobayashi, K. Tsukanoto, V. Hearing, J. Biol. Chem. 268 (1993) 25650.
[8] M. Wick, J. Invest. Dermatol. 74 (1980) 63.
[9] H. Hara, N. Walsh, K. Yamada, K. Jimbow, J. Invest. Dermatol. 102 (1994) 501.
[10] B. Faraj, V. Camp, D. Murray, M. Kutner, J. Hearn, D. Nixon, Clin. Chem. 32 (1986) 159.
[11] K. Yamada, N. Walsh, H. Hiroyuki, K. Jimbow, H. Chen, S. Ito, Arch. Dermatol. 128 (1992) 491.
[12] B. Sonesson, S. Eide, U. Ringborg, H. Rorsman and E. Rosengren, Melanoma Res., 5 (1995) 113.
[13] S. Sarre, J. Lojewski, C. Hiemke, D. Deleu, N. Deklipell, G. Ebinger, J. Chromatogr. 575 (1992) 207.
[14] J.R. Lee Chin, J. Chromatogr. 578 (1992) 17.
[15] V. Rizzo, G. Melzi d'eric, G. Pierocellerino, J. Chromatogr. 536 (1991) 229.
[16] A. Jussofie, J. Lojewski, C. Hiemke, J. Liq. Chromatogr. 16 (1993) 447.
[17] I. Rondelli, D. Acerbi, F. Mariotti, P. Ventura, J. Chromatogr. B 653 (1994) 17.
[18] R.B. Miller, L. Dehelean, L. Belanger, Chromatographia 35 (1993) 9.
[19] J. Dutton, L.G. Copeland, J.R. Playfer, N.B. Roberts, Clin. Chem. 39 (1993) 629.
[20] M. Ruhul Amin, Y. Tomita, R. Onodera, J. Chromatogr. B 663 (1995) 201.
[21] J. Rudy, J. Rutledge, S. Lewis, Clin. Chem. 33 (1987) 1152.
[22] S. Ishimitsu, S. Fujimoto, A. Ohara, J. Chromatogr. 378 (1986) 222.
[23] A.H. Anton, D.F. Sayre, J. Pharmacol. Exp. Ther. 138 (1962) 360.
[24] C. Holmes, G. Eisenhofer, D. Golstein, J. Chromatogr. B 653 (1994) 131.
[25] L. Peyrin, R. Mornex, Ann. Biol. Clin. 50 (1992) 835.
[26] P. Bouloux, D. Perrett, G.M. Besser, Ann. Clin. Biochem. 22 (1985) 194.
[27] C. Luradcelli, P. Betto, M. Giambenedetti, G. Ricciarello, S. Ruggeri, F. Stocchi, Giorn. It. Chim. Clin. 12 (1987) 223.