

DEMONSTRATION OF 3-METHOXYTYROSINE IN THE URINE OF MELANOMA PATIENTS

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Abstract. With the aid of gas chromatography and mass spectrometry a metabolite of dopa, 3-methoxytyrosine, has been demonstrated in the urine of 2 patients with melanoma metastases, the amounts excreted being 4.0 mg and 8.5 mg/24 hours, respectively. 3-Methoxytyrosine could not with certainty be identified in the urines of two normal subjects.

Key words: Melanoma; Methoxytyrosine; Amino acids

Certain melanoma patients excrete specific melanoma metabolites in the urine in such large amounts that a laboratory diagnosis of melanoma is possible. Traditionally, Thormählen-positive melanogens have received much attention in the investigation of patients with melanoma. Quantitative determination of Thormählen-positive melanogens has practically no value in early diagnosis, however (5). The determination of dopa, which is an intermediate substance in melanin formation, has been considered to be of greater value (7, 11, 12, 13). Recent studies have indicated that assessment of another amino acid, 5-S-cysteinyldopa, which is a specific metabolite of the melanocytes, yields even more information (1).

It now seems possible to obtain a more thorough understanding of the biochemistry of melanin formation in melanoma cells by studying the specific intermediate products, some of which may have cytotoxic properties (8). Practically nothing is at present known about whether certain metabolic differences in melanin formation may be related to other features of melanomas. The detection of specific melanoma metabolites and the development of quantitative methods for their investigation will probably make it possible in the near future to

define a biochemical profile of every melanoma. It will then become necessary to investigate to what extent differences in melanogenesis may be correlated to other parameters of melanoma, e.g. growth and immunogenesis. •

As a basis for this a definition of the compounds observed in pathological amounts in the urine of melanoma patients will be necessary. The detection of a new substance in the urine of melanoma patients, 3-methoxytyrosine, is reported here.

MATERIAL AND METHODS

24-hour specimens of urine were collected from 2 healthy adults, one man and one woman, and from 2 men aged 46 and 64 years (H. N. and C. R.) with widespread melanoma metastases and general increase in pigmentation.

The specimens were collected in plastic bottles containing 50 ml glacial acetic acid and 1 g sodium metabisulphite. Dopa+dopamine and 5-S-cysteinyldopa were determined by methods previously described (3, 10).

20-ml specimens of urine were put on a column measuring 27×5 mm and containing Dowex 50W-X4, H⁺-form. Elution was performed with 2 N HCl, and the effluent was collected in 5-ml fractions. The second fraction was further investigated by combined gas chromatography and mass spectrometry (GC-MS).

For this purpose 1 ml of the second fraction was evaporated to dryness and the residue dissolved in 150 µl methanol-HCl, prepared by passing HCl gas, passed through H₂SO₄, into methanol to a concentration of 3 N HCl. The sample was then heated for 15 min at 100°C in a sealed tube. Next, the methanol-HCl was evaporated using a stream of dry nitrogen. 30 µl pentafluoropropionic anhydride (PFPA) was then added and the tube heated at 60°C for 30 minutes in the tapered reaction vial. The PFPA was evaporated with dry nitrogen and the residue dissolved in 30 µl 1% PFPA in ethyl acetate.

Authentic 3-methoxytyrosine was treated with methanol-HCl and PFPA in the same way as the speci-

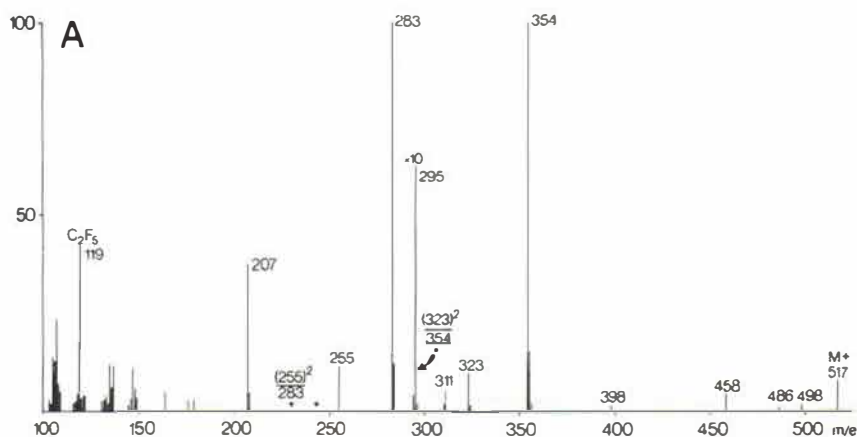
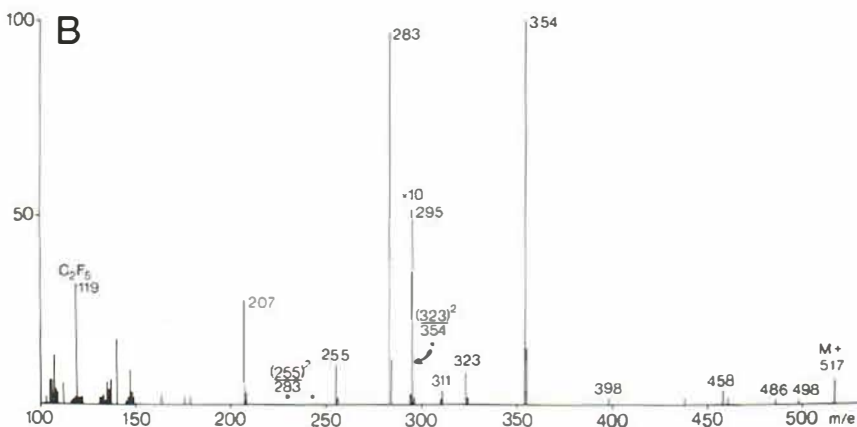
RELATIVE
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Fig. 1. Mass spectrogram of PFPA-methyl-derivative of 3-methoxytyrosine (A) and of the compound in melanoma urine (B).

mens of urine. The gas chromatograph mass spectrometer used was an LKB 2091. For gas chromatography a glass column (9 ft $\times$ 2 mm) containing 3% OV-17 on Chromosorb W, mesh 100–120, was used. The working conditions were as follows.

Column temperature 180°C. Flash heater 220°C. Helium flow 20 ml/min. Temperature of the molecular separator of the mass spectrometer 240°C. Ion source temperature 270°C. Ionizing voltage 70 eV. Standard accelerating voltage 3.5 kV. Trap current 50 μ Amp.

For quantitation of 3-methoxytyrosine in the eluates, the ion intensity of three fragments, i.e. at *m/e* 354, 295 and 283, was measured by mass fragmentography using 1–2 μ l of eluate from melanoma patients and 25 μ l of eluate from healthy subjects. α -methyl-*p*-tyrosine treated with methanol-HCl and PFPA was used as internal standard (*m/e* 338).

RESULTS

Gas chromatography combined with mass spectrometry of material isolated from the melanoma urines showed the presence of a substance with the same retention time and the same fragmentation pattern as the 3-methoxytyrosine derivative (Fig. 1). Fragmentation of the 3-methoxytyrosine derivative takes place in all probability as illustrated in Fig. 2. Some of the fragments were established by esterification with deuterized methanol.

Quantitation of 3-methoxytyrosine was performed by mass fragmentography. Of several internal standards considered, α -methyl-*p*-tyrosine

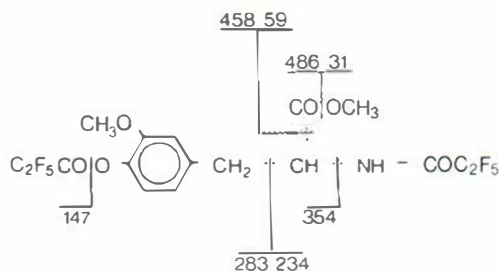


Fig. 2. Fragmentation of PFPA-methyl-derivative of 3-methoxytyrosine.

was found the most satisfactory. The urines investigated were tested for the presence of a substance with the same retention time as α -methyl-*p*-tyrosine and with a fragment at *m/e* 338. No substance fulfilling these criteria was found when the above amounts of the eluate were used. The retention time for 3-methoxytyrosine was 4.92 min and for α -methyl-*p*-tyrosine, 2.28 min. The fragments of the methoxy derivative at *m/e* 354, 295, and 283 were used. The same ratios of intensities of the fragments at *m/e* 354, 295, and 283 were obtained for the derivative from melanoma urines as for the authentic 3-methoxytyrosine (Fig. 3). It should be noted that relative intensities of the

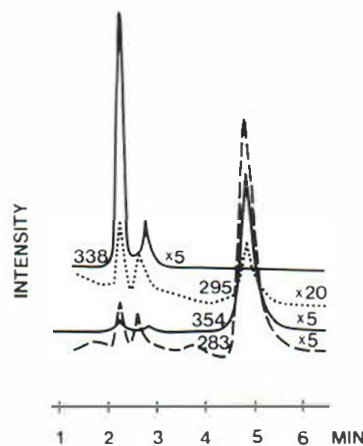


Fig. 4. Mass fragmentogram obtained from analysis of a melanoma urine. Internal standard (α -methyl-*p*-tyrosine *m/e* 338).

fragments in Fig. 1 are not the same as those in Fig. 3. This is due to the fact that the values in Fig. 1 were obtained with constant accelerating voltage and varying magnetic field, whereas the data in Fig. 3 were obtained with varying accelerating voltage and constant magnetic field.

The mass fragmentogram of one of the melanoma urines is shown in Fig. 4. The urinary excretion of 3-methoxytyrosine is given in Table I, which also shows the excretion of 5-S-cysteinyl-dopa and of dopa+dopamine.

Patient H. N. with melanoma excreted 4.0 mg of 3-methoxytyrosine per 24 hours, and patient C. R. 8.5 mg/24 hours.

The mass spectrum obtained from processed urines of healthy subjects has not produced definite proof of 3-methoxytyrosine, since the ratios between the ion densities of the fragments selected for

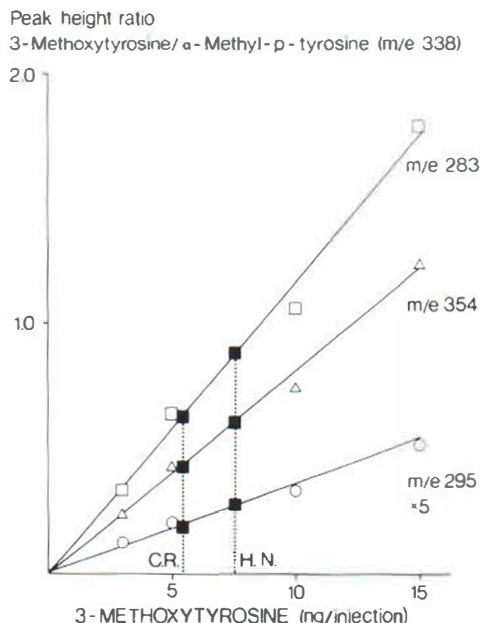


Fig. 3. Mass fragmentometric analysis of 3-methoxytyrosine in melanoma urines.

Table I. Urinary excretion of 3-methoxytyrosine, dopa+dopamine, and 5-S-cysteinyl-dopa in 2 healthy subjects and in 2 patients with melanoma metastases (mg/24 hours)

	Healthy subjects		Melanoma patients	
	L. E.	K. P.	H. N.	C. R.
3-methoxytyrosine	<0.11	<0.09	4.0	8.5
Dopa+dopamine	0.25	0.18	1.6	5.6
5-S-cysteinyl-dopa	0.29	0.07	57	28

mass fragmentography were not identical to those of the 3-methoxytyrosine derivative. It may be concluded that the excretions of 3-methoxytyrosine in the 2 healthy subjects were less than 0.09 and 0.11 mg per 24 hours, respectively.

The excretion of dopa+dopamine in patient H. N. with melanoma metastasis was 1.6 mg/24 hours, in patient C. R. 5.6 mg/24 hours, and in the 2 healthy subjects 0.25 mg and 0.18 mg/24 hours, respectively. Patient H. N. with melanoma metastasis excreted 57 mg 5-S-cysteinyl-dopa per 24 hours and patient C. R. 28 mg/24 hours. The amounts of 5-S-cysteinyl-dopa were 0.29 mg and 0.07 mg/24 hours in the 2 healthy subjects.

DISCUSSION

Dopa, a key substance in melanization, is metabolized in the body to a large number of compounds (6). One of the principal metabolites is 3-methoxytyrosine. The amount of this substance excreted in the urine may reflect the dopa that has reached the circulation; but 3-methoxytyrosine may also be formed in dopa-containing cells which contain catechol-O-methyl transferase. In the urine from melanoma patients 3-methoxytyrosine was present in substantially larger amounts than was dopa. This raises the interesting possibility that determination of 3-methoxytyrosine in the urine of patients with melanoma may prove important in the investigation of the catechol metabolism of patients with this condition.

It has previously been reported that the urinary excretion of a recently detected amino acid, 5-S-cysteinyl-dopa, sensitively reflects the pigment formation (9). 5-S-cysteinyl-dopa is the result of interaction of dopaquinone with cysteine or glutathione in the melanocytes (2).

In contrast, the excretion of 3-methoxytyrosine may reflect the amount of non-oxidized dopa or 3-methoxytyrosine leaking from the melanocyte, and perhaps also from other cells. If 3-methoxytyrosine has its origin in the melanocytes alone, simultaneous determination of 5-S-cysteinyl-dopa and 3-methoxytyrosine in the urine will yield important information on the catechol metabolism of the pigment-forming cell.

Using a recently reported method, Banda et al. have analysed reducing compounds in the urine of melanoma patients, and have found a great number

of unidentified substances, one of which shows the chromatographic behaviour of 3-methoxytyrosine (4). We have used different chromatographic systems, and the present finding of 3-methoxytyrosine and the previous one of 5-S-cysteinyl-dopa in melanoma urines (1) do not permit of any conclusion with regard to the identity of the peaks reported by Banda et al. (4).

ACKNOWLEDGEMENTS

This investigation has been supported by grants from the Swedish Cancer Society, the Swedish Medical Research Council, the Walter, Ellen and Lennart Hesselman Foundation for Scientific Research, and the John and Augusta Persson Foundation for Scientific Medical Research.

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Received March 17, 1975

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