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URINARY EXCRETION OF 5-S-CYSTEINYLDOPA IN PATIENTS WITH PRIMARY MELANOMA OR MELANOMA METASTASIS

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Abstract. Urinary excretion of 5-S-cysteinyldopa was studied in 9 patients with primary melanoma. All had 5-S-cysteinyldopa excretion in the normal range. In 8 of the patients excretion values decreased after removal of the tumour. Twenty-four patients with clinical signs of melanoma metastasis were examined for 5-S-cysteinyldopa and dopa + dopamine in the urine. 16 of the 24 had pathologically increased excretion of 5-S-cysteinyldopa and 7 of the 24 had pathological excretion of dopa + dopamine. Six of the latter belonged to the group with increased 5-S-cysteinyldopa excretion and one patient had a borderline value of 5-S-cysteinyldopa. Determination of 5-S-cysteinyldopa seems to be of value in the follow-up of patients operated on for primary melanoma.

Key words: Dopa; Dopamine; Aminoacids; Catechol; 5-S-cysteinyldopa; Melanoma

The amino acid, 5-S-cysteinyldopa, has newly been detected in human melanomas (1, 5, 15). This amino acid was synthesized by Prota et al. (10) who found that 5-S-cysteinyldopa was an intermediate substance in the biogenesis of phaeomelanins.

Two years ago we observed a high amount of 5-S-cysteinyldopa in the urine of some melanoma patients (7). The amino acid has also been found regularly in small amounts in the urine of healthy subjects, irrespective of their hair colour (2). We have now systematically examined urine of melanoma patients for 5-S-cysteinyldopa.

MATERIAL AND METHODS

Urine was collected for 24 hours from 76 healthy Caucasians, 30 men and 46 women, aged 18 to 91 years. This material has been previously published (2).

9 patients, 5 men and 4 women, with primary melanoma of the skin but without signs of metastases were studied before and within one week after operation for their melanomas. Sample volumes were analysed in these cases and 5-S-cysteinyldopa/creatinine excretion ratios were calculated.

24 patients, 16 men and 8 women, who were previously operated on for malignant melanomas and who were found to have metastases, were studied with regard to their excretion of 5-S-cysteinyldopa and dopa + dopamine. In all these cases, urine was collected for 24 hours. Urine was collected in plastic bottles containing 50 ml acetic acid and 1 g sodium metabisulphite. 20 ml of the collected urine was adsorbed to Al_2O_3 and eluted with 0.1 N HCl. Dopa + dopamine and 5-S-cysteinyldopa were determined fluorimetrically by methods described previously (4, 11). Creatinine in urine was determined as described by Clark & Thompson (6).

RESULTS

Healthy controls. The amount of 5-S-cysteinyldopa in the urine of 76 healthy subjects varied between 9 and 240 μg/24 h (2) corresponding to 4–280 ng/ml. The ratio of 5-S-cysteinyldopa excretion (ng) and creatinine excretion (mg) varied between 10 and 170. Dopa + dopamine varied between 73 and 520 μg/24 h.

Patients with primary melanoma. It is evident from Fig. 1 that the ratios of 5-S-cysteinyldopa/creatinine were within the normal range in the patients with primary melanoma but who had no signs of metastasis. The ratios were lower after surgery in 8 of the 9 patients studied.

Patients with metastasis. The findings in patients with metastases are presented in Fig. 2. 16 of the 24 had pathologically increased values of 5-S-cysteinyldopa, while 8 patients had values within the normal range. An account of the 5-S-cysteinyldopa excretion is given in Table I, which also shows the results of the dopa + dopamine determinations. Seven of the 24 patients had pathological excretion of dopa + dopamine. All these patients had considerable amounts of 5-S-cysteinyldopa in the urine and in 6 of the patients the 5-S-cysteinyldopa values

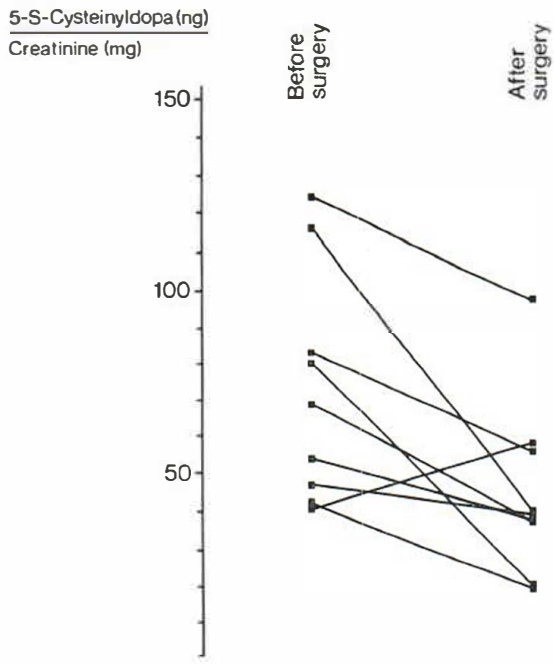


Fig. 1. Excretion ratio of 5-S-cysteinyldopa (ng)/creatinine (mg) in patients with primary melanoma.

were definitely pathological. Age and hair colour did not seem to influence the amount of 5-S-cysteinyldopa excretion in patients with metastases.

13 of the 16 men had pathological 5-S-cysteinyldopa excretion, whereas only 3 of the 8 women did so. 4 of the women had non-pigmented or faintly pigmented metastases.

In the presence of metastases, 5-S-cysteinyldopa excretion was positive, irrespective of the origin of the primary melanoma.

Table II shows the development of 5-S-cysteinyldopa excretion in a patient with advanced dissemination of melanoma metastases. The progressive increase of 5-S-cysteinyldopa excretion is not correlated with a corresponding increase of dopa + dopamine.

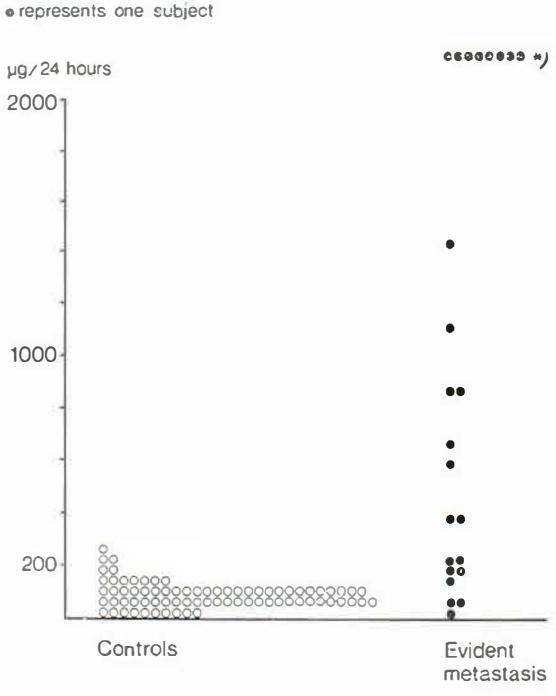
DISCUSSION

The excretion of 5-S-cysteinyldopa was in the normal range in the patients with primary melanoma. However, after removal of the tumour, the values decreased in most patients. The excretion of 5-S-cysteinyldopa after surgery should be considered to be the "normal" value of the patient when checked for metastasis by 5-S-cysteinyldopa determinations.

The present investigation has shown that there is a good correlation between 5-S-cysteinyldopa in the urine and the occurrence of pigmented melanoma metastasis.

There are many previous reports on the occurrence of "melanogens" or metabolites in the urine of melanoma patients. Traditional investigations of melanogens such as the Thormählen reaction are insensitive and become positive only in patients with advanced disease (8). The same holds true for determinations of dopamine and dopamine metabolites such as homovanillic acid and 3,4-dihydroxyphenylacetic acid (dopac) (9, 16).

The amino acid that until now has been most specifically linked to melanin metabolism is dopa. This substance has often been found in increased amounts in the urine of patients with melanomas (9, 13, 14, 16). Methods used in studies on dopa, before the detection of 5-S-cysteinyldopa, must be checked for specificity with regard to the two different amino acids. The relative value of urinary dopa and 5-S-cysteinyldopa in the follow-up of melanoma patients remains to be investigated.



*) the individual values in these 8 patients were 3000, 3600, 4300, 6000, 16000, 17000, 25000 and 28000 µg/24 hours.

Fig. 2. 5-S-cysteinyldopa in the urine of controls and of melanoma patients.

Table I. *5-S-cysteinyldopa and dopa + dopamine in the urine of patients with melanoma metastasis*

	Age	Sex	Hair colour	Primary melanoma	Metastasis	5-S-cysteinyl-dopa (μg/24 h)	Dopa + dopamine (μg/24 h)
1.	42	♀	Sandy	Skin	Regional lymph node Faintly pigmented	39	63
2.	84	♀	Grey	Skin	Two-subcutaneous Non-pigmented	69	150
3.	78	♀	Grey	Skin	Reg. and dist. l.n. Faintly pigmented	74	150
4.	51	♂	Red	Skin	Reg. l.n.	150	370
5.	61	♀	Red	Skin	Multiple subcutaneous Weakly pigmented	190	310
6.	76	♂	Blonde	Skin	Local cutaneous, l.n., pulmonary, hepatic Faintly pigmented	200	170
7.	59	♂	Brown	Unknown	Two subcutaneous, cerebral	210	170
8.	49	♀	Brown	Skin	Reg. l.n.	230	610
9.	68	♂	Brown	Skin	Reg. l.n. + cutaneous	360	220
10.	46	♂	Brown	Skin	Reg. l.n., cerebral	400	200
11.	68	♂	Blonde	Skin	Solitary distant l.n.	600	390
12.	74	♂	White	Nasal mucosa	Gastrointestinal	650	150
13.	43	♀	Red	Eye	Multiple subcutaneous, dist. l.n.	870	500
14.	55	♂	Grey	Skin	Reg. l.n.	870	510
15.	82	♂	Grey	Skin	Reg. l.n.	1 100	240
16.	57	♂	Red	Skin	Hepatic	1 400	140
17.	54	♂	Unknown	Eye	Skeletal, hepatic	3 000	1 900
18.	68	♀	White	Genital mucosa	Pulmonal, cerebral	3 600	1 900
19.	73	♂	Grey	Skin	Reg. l.n., multiple cutaneous	4 300	520
20.	40	♂	Red	Skin	Hepatic	6 000	400
21.	33	♂	Sandy	Skin	Mult. cutaneous-subcutaneous pulmonary	16 000	910
22.	25	♂	Blonde	Skin	Mult. subcutaneous	17 000	2 200
23.	66	♀	Unknown	Eye	Skeletal, pulmonary	25 000	1 700
24.	44	♂	Brown	Unknown	Hepatic	28 000	6 300

Determination of the sum of the two catechols dopa and dopamine, as carried out on urine of all patients in the present study, gives poor information on the extent of the dissemination of melanomas. This is easily understood, since dopa and dopamine are also formed outside the melanin-forming cells.

There is evidence that the excretion of 5-S-cysteinyldopa specifically reflects functions of the melanocytes. Sun exposure inducing pigment formation is followed by increased 5-S-cysteinyldopa excretion (12). Decrease in pigmentation in the more elderly is correlated to excretion of 5-S-cysteinyldopa (2).

The majority of the patients with melanoma metastases have higher values of 5-S-cysteinyldopa in the urine than normal subjects. However, there is also some overlapping of values in the groups; 8 out of 24 patients had excretion values in the normal range. 4 of these patients had widespread metastases that were faintly pigmented, 3 patients had small regional metastases with moderate pigment content, and one patient had non-pigmented metastases. Thus, a normal 5-S-cysteinyldopa value never excludes dissemination of a melanoma, since the excretion of this amino acid is related to the pigmentation of the tumour. A high 5-S-cysteinyldopa value in a patient investigated for melanoma metastasis is of great significance, but again it should be remarked that 5-S-cysteinyldopa excretion reflects not malignancy but melanocyte function. Exposure to sunlight may lead to 5-S-cysteinyldopa values in the urine several times higher than pre-exposure values (12).

A chemical method for the detection of melanoma metastasis should ideally give pathologic values before there are evident clinical signs of spreading, in

Table II. *5-S-cysteinyldopa and dopa + dopamine excretion in a patient with progressing melanoma metastases (No. 22 in Table I)*

Age	Sex	Hair colour	Clinical history	Date	5-S-cysteinyl-dopa (μg/24 h)	Dopa + dopamine (μg/24 h)
25	♂	Blonde	Primary melanoma of the back and hilat. axillar l.n. metastases	June 1973		
			Local cutaneous m.	Oct. 1973		
			Multiple subcutaneous m.	Nov. 1973	17 000	2 200
			Mult. dist. l.n. m.	Jan. 1974	29 000	1 000
			Hepatic m.	May 1974	43 000	1 900
			Death	Sept. 1974	110 000	1 900
			Autopsy: Disseminated pigmented melanoma metastases			

order to give surgery, chemotherapy or immunotherapy a better chance. Previously used chemical methods for the diagnosis of melanoma metastasis do not seem to have detected metastases before they became clinically evident. In the series of melanomas presented here, clinical signs of melanoma spread were present in all cases. The early detection of spreading melanomas can therefore not be evaluated on the basis of this material. However, in a follow-up study of patients operated on for primary melanomas, increase in 5-S-cysteinyldopa was often the first sign of metastases. In several cases abnormal 5-S-cysteinyldopa findings directly initiated investigations leading to the detection of melanoma metastases (3).

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