

Evaluation of the Clinical Usefulness of Measuring Urinary Excretion of 5-S-cysteinyl-dopa in Melanoma: Ten Years' Experience of 50 Patients

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The urinary excretion of 5-S-cysteinyl-dopa (5-S-CD) is known to be increased in certain patients with melanoma. To evaluate its diagnostic and prognostic utility, we measured the urinary excretion of 5-S-CD on at least three different occasions in 50 patients with melanoma. No significant increase was found in 26 patients without metastases, in 10 patients with regional lymph node metastasis and 2 patients with amelanotic melanoma. However, all the 12 patients with distant metastases demonstrated a significant increase. The patients with 5-S-CD > 1,000 µg/day survived for a mean of 8.1 ± 5.6 months, while those with 5-S-CD > 10,000 µg/day survived for 3.5 ± 3.7 months. All the 4 patients with a maximum excretion of 5-S-CD > 40,000 µg/day had multiple liver metastases. In conclusion, while data on the urinary excretion of 5-S-CD was not useful in the detection of early regional lymph node metastases, its increase indicated the presence of distant metastases and also provided prognostic information. **Key words:** malignant melanoma; precursor of pheomelanin; metastasis; prognosis.

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Normal melanocytes, as well as melanoma cells, produce two types of melanin pigment, black eumelanin and reddish brown pheomelanin. Levels of 5-S-cysteinyl-dopa (5-S-CD), a precursor of pheomelanin (1), have been reported to be increased in the urine (2), sera (3) and tumors (4) of patients with melanoma. Measurements of 5-S-CD in urine and serum have therefore been used to evaluate disease activity in patients with malignant melanoma (4, 5). Since the increased urinary excretion of 5-S-CD in patients with melanoma was first reported in 1973 (2), many investigators have demonstrated its elevated excretion in metastatic melanoma (4–9). However, there are few studies in which the relationship between the excretion of 5-S-CD, results of radiological investigations, and the clinical course are objectively compared in a large number of patients over a long period. The early detection of metastatic melanoma is important in selecting the appropriate treatment, in improving the patient's clinical status, and in predicting survival. We therefore compared the diagnostic utility of measuring the urinary excretion of 5-S-CD with that of radiographic investigation and physical examination in patients with malignant melanoma.

MATERIAL AND METHODS

Patients

The present investigation was conducted between 1984 and 1995, in 116 Japanese patients with melanoma of the skin, eye, mucosa, or a

melanoma that had metastasized from an unknown primary site. In that period, we examined a total of 1,270 samples for the measurement of daily urinary excretion of 5-S-CD. Out of 116 patients, 50 patients whose urinary excretion of 5-S-CD was examined at least three times during their clinical course were utilized for evaluation. They included 30 males and 20 females, age range 19 to 77 years (mean 50.7 ± 18.8 years). The mean follow-up period was 5.5 years (range 0.5 to 10 years). Patients were clinically evaluated at least every 6 months, by means of physical examination and radiological investigation. The 50 patients were classified into five groups, as follows: without metastasis ($n=26$; 11 men and 15 women, mean age 45.3 years); with regional lymph node metastasis ($n=10$; 5 men and 5 women, mean age 37.2 years); with distant metastasis ($n=12$; 8 men and 4 women, mean age 66 years) and amelanotic melanoma ($n=2$; 2 women, mean age 57 years). The clinical types included 19 cases of acral lentiginous melanoma, 20 nodular melanomas, 7 superficially spreading melanomas, 1 lentigo malignant melanoma and 3 unclassified melanomas. Ten healthy Japanese (5 men and 5 women, mean age 50 years) served as controls. Their daily urinary excretion of 5-S-CD was evaluated. Although previous studies avoided exposing the patients to strong sunlight for 1 month before the evaluation of urinary 5-S-CD, this was not attempted in the present study.

Measurement of urinary excretion of 5-S-CD

The urinary level of 5-S-CD was determined by high-performance liquid chromatography with electrochemical detection using a borate column, according to a modified version of a method previously reported (10–12). We used the following devices in the measurement of 5-S-CD: a model CCPM computer-controlled multipump (Toso); a Model PT-8000 (Toso) column-switching valve system; a borate column (precolumn TSK gel B-3PW (Toso); an ODS cartridge column (SSC-2151-Y 0.6X15cm, Sensyu Kagaku); and an electrochemical detector (ECD: VMD EC-8, Volumetric detector Yanako with DC-100 high-sensitivity cell). Twenty-four-hour urine samples were collected in plastic bottles, which contained 50 ml acetic acid and 1 g sodium metabisulphite. A volume of 10 ml of the collected urine was frozen at -20°C until use. A volume of 1 ml urine was mixed with 1 ml of 1 mol/l dipotassium phosphate (pH 8.6), poured onto the loop injector and introduced into a borate column. The 5-S-CD fraction was absorbed in the borate column, then eluted with 0.1 mol/l monobasic potassium phosphate (PH1.7), and the eluate was introduced automatically into an ODS column. The 5-S-CD fraction was quantified with an electrochemical detector (ECD). The conditions for ECD were as follows: the detector potential was set at 0.5 V against Ox, 1 nA range 1/4, and chart speed 0.5 cm/min. The urinary levels of 5-S-CD were interpreted as follows: normal, below 400 µg/day, and abnormal, over 400 µg/day. The mean urinary excretion of 5-S-CD in the control subjects was 152 ± 121 µg/day. Statistical analysis was performed using Fisher's exact test. A level of $p < 0.05$ was accepted as statistically significant.

RESULTS

There was no significant increase in the urinary excretion of 5-S-CD in the 26 patients without metastasis ($p=0.7439$, mean \pm SD 165 ± 70 µg/day, range 93 to 310 µg/day), in 10 patients with regional lymph node ($p=0.1678$, mean \pm SD

Table I. Urinary excretion of 5-S-CD in melanoma patients

LN: lymph node

Patients' status	Total urinary excretion of 5-S-CD ($\mu\text{g}/\text{day}$)				
	Number of cases	<400*	400–1000*	1000–10000*	10000 >
No metastasis	26	26	0	0	0
Regional LN metastasis	10	10	0	0	0
Distant metastasis	12	0	0	5	7
Amelanotic melanoma	2	2	0	0	0

202 \pm 67 $\mu\text{g}/\text{day}$, range 146 to 292 $\mu\text{g}/\text{day}$), or in 2 patients with amelanotic melanoma with distant metastasis ($p=0.8218$, mean \pm SD 136 \pm 20 $\mu\text{g}/\text{day}$, range 158 to 127 $\mu\text{g}/\text{day}$) (Table I). In the 26 patients without metastasis, the mean value of the urinary excretion of 5-S-CD of men ($n=11$) and women ($n=15$) was 168 \pm 20 $\mu\text{g}/\text{day}$, 150 \pm 15 $\mu\text{g}/\text{day}$ (mean \pm SD) respectively, and there was no significant difference ($p=0.2418$).

Of the 12 patients with distant metastases, 5 patients demonstrated such metastases on their first visit. The remaining 7 patients, who developed metastases, exhibited a significant increase in the urinary excretion of 5-S-CD ($p=0.007$, mean \pm SD 693 \pm 182 $\mu\text{g}/\text{day}$, range 444 to 850 $\mu\text{g}/\text{day}$), apparently earlier (mean \pm SD 3.0 \pm 2.9 months, range 1 to 14 months) than had been detected by radiological examination. Multiple liver metastases were found in 4 patients, all of whom exhibited an elevated urinary excretion of 5-S-CD above 40,000 $\mu\text{g}/\text{day}$ (mean \pm SD 81,539 \pm 55,003 $\mu\text{g}/\text{day}$, range 40,300 to 162,500 $\mu\text{g}/\text{day}$). Their mean duration of survival after their urinary excretion of 5-S-CD rose above 1,000 $\mu\text{g}/\text{day}$ ($n=5$) was 8.1 \pm 5.6 months (range 2 to 14 months), and that above 10,000 $\mu\text{g}/\text{day}$ ($n=7$) was 3.5 \pm 3.7 months (range 1 to 10 months).

DISCUSSION

The present study showed that a significant increase in the urinary excretion of 5-S-CD in patients with melanoma provided evidence of distant metastasis. However, this test was not useful for the early detection of regional lymph node metastases or distant metastasis in patients with amelanotic melanoma. None of the 10 patients with early regional lymph node metastasis exhibited a significant increase in the urinary excretion of 5-S-CD or any abnormal radiological findings on CT and gallium scans. Physical examination and lymph node biopsy provided early evidence of regional lymph node metastases.

In a previous study, excretion of 5-S-CD below 150 $\mu\text{g}/\text{day}$ was regarded as normal, between 150 $\mu\text{g}/\text{day}$ and 400 $\mu\text{g}/\text{day}$ was regarded as borderline, and above 400 $\mu\text{g}/\text{day}$ as abnormal (9). In our study, the mean urinary excretion of this substance in healthy subjects was 152 \pm 121 $\mu\text{g}/\text{day}$, similar to the 141 to 296 $\mu\text{g}/\text{day}$ reported in other studies of healthy controls (13). The urinary excretion of 5-S-CD, when distant metastasis was detected, exceeded 400 $\mu\text{g}/\text{day}$ (range 444–820 $\mu\text{g}/\text{day}$); thus this level was considered as abnormal in the present study. Although previous studies by other investigators avoided exposing the patient to strong sunlight for 1 month before the evaluation of urinary 5-S-CD (4, 9), this limitation is difficult

to define as well as to ensure, and it was not attempted in the present study. Protection from the sun was not required for detecting metastasis by abnormal urinary excretion of 5-S-CD. The frequent measurements of 5-S-CD may be more important.

It is not clear why 4 patients with multiple liver metastasis in the present study demonstrated extremely high urinary levels of 5-S-CD, above 40,000 $\mu\text{g}/\text{day}$ (mean 81,538 $\mu\text{g}/\text{day}$). Our data suggest that a marked increase in the urinary excretion of 5-S-CD is a sign of multiple liver metastasis. Our study also demonstrated that urinary excretion of 5-S-CD over 1,000 $\mu\text{g}/\text{day}$ can provide prognostic information. Plasma levels of 5-S-CD are reportedly useful in detecting metastasis in patients with melanoma (3, 5). Since plasma levels of this substance are relatively low and difficult to detect, the evaluation of the urinary excretion of 5-S-CD seems to offer a clinical advantage in detecting patients with lymph node metastases. In conclusion, although the urinary excretion of 5-S-CD was not useful in detecting early regional lymph node metastasis of melanoma or the spread of amelanotic melanoma, this value reflects the presence of distant metastases and provides clinical information related to prognosis.

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