

URINARY 5-S-CYSTEINYLDOPA IN PARKINSONISM AFTER DOPA AND CARBIDOPA

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Abstract. The effect of anti-Parkinson therapy on the urinary excretion of 5-S-Cysteinyldopa (5SCD), a catechol metabolite of dihydroxyphenylalanine (DOPA) and marker for the melanocyte, was studied by means of high performance liquid chromatography. 5SCD was normal in Parkinson patients treated with anticholinergics. DOPA administration increased 5SCD excretion. Carbidopa and DOPA together elevated 5SCD markedly in a dose-dependent manner to values higher than seen in some patients with metastatic malignant melanoma. The effect of anti-Parkinson therapy should be considered when using 5SCD as a tumor marker or when a Parkinson patient has a melanoma.

Key words: Melanoma; Cysteinyldopa; DOPA; Carbidopa; Parkinsonism

Concern and controversy has arisen over what risk, if any, long-term administration of levodopa (DOPA) for Parkinsonism poses for developing malignant melanoma (1-7). While the risk appears to be exceedingly small (4), several cases of malignant melanoma, including 2 cases of non-familial multiple primary melanomas, have been reported in patients who had received levodopa (1, 7). Although a clear relationship for melanoma induction has not been established, DOPA is a well known biosynthetic precursor of melanin, and the urinary excretion of DOPA has been found to be elevated in a few cases of malignant melanoma (8). More recently, a catechol metabolite of DOPA, 5-S-Cysteinyldopa (5SCD) has been found to be elevated in the urine of many patients with metastatic pigmented melanoma (9-12). Since 5SCD appears to be an important marker for melanocyte activity, we evaluated the effect of DOPA and carbidopa on 5SCD metabolism in Parkinson patients without melanoma.

MATERIALS AND METHODS

Patient selection

We studied 40 Parkinson out-patients of different ages (35-75 yrs), disease duration (1-27 yrs) and severity of

illness (Stage I-Stage V) (13). Treatment included no medication, anticholinergics, or varying amounts of DOPA, either alone or DOPA plus carbidopa. Most patients had been receiving DOPA plus carbidopa for more than 5 years. No Parkinson patients were receiving vitamin B₆ or had skin lesions suggestive of melanoma. Controls were selected from both normal individuals of comparable age as well as out-patients with retrocollis, torticollis, or paraplegia. The urine from 8 additional non-hypertensive, non-neurological in-patients was also pooled and evaluated. Three patients with melanoma were also evaluated.

Urine collection

A 24-hour urine sample was collected from out-patients after 3 days of a low "VMA" diet which excluded coffee, tea, soft drinks, chocolate, licorice, mature cheese, bananas, and vanilla. Dietary compliance was ascertained by patient and spouse report. The collection bottle contained 25 ml of 6 M HCl. The final urine pH after collection was adjusted to 2.0. Total volumes were measured and several 12-ml aliquots were stored at -70°C until analysed. Reanalysis of samples stored under these conditions showed stable levels of DA, DOPA, and 5SCD for at least a year.

Catechol analysis

3-ml samples of filtered urine containing isoproterenol (IPT) as an internal standard were deproteinized with 2 g NH₄SO₄ (14, 15). The supernatant was washed twice with 5 ml ethyl acetate and once with 5 ml hexane. The aqueous phase was transferred to a 10 ml beaker and 100 µl of 10% EDTA solution and 100 µl of 5% Na₂S₂O₅ solution were added. The samples were rapidly adjusted to pH 8.4-8.5 with approximately 5 drops of 3 N NaOH and immediately bound to 70 mg of alumina. They were washed three times with phosphate buffer, pH 7.4, and dried in a vacuum oven. The catechols were eluted with 1.8 ml 1 N perchloric acid. The eluates were filtered before analysis on a high performance liquid chromatograph (Perkin Elmer) employing a LC₁ electrochemical detector (Bioanalytical Systems). The mobile phase buffer consisting of 2.9 g phosphoric acid and 6 g methanesulfonic acid at pH 1.75 ran at a flow rate of 0.8 ml/min. The reference electrode was set at 0.75 mV. Samples (100 µl) were injected onto a 5µ C₁₈ Nucleosil column. Authentic standards of DOPA and DA were obtained from Sigma. 5SCD was a gift from Professor Agrup of University of Lund, Sweden (9). Peak areas were integrated using a Perkin

Table 1. Urinary catechols in Parkinsonism (PD)

Condition (N)	Oral (mg/day) Carbidopa/ DOPA	Urine ($\mu\text{g/day}$) \pm (SD)		
		DOPA	DA	5SCD
Urine pool (22)	0/0	23 \pm 9	117 \pm 29	81 \pm 33
Controls (10)	0/0	34 \pm 38	154 \pm 94	83 \pm 91
Melanoma (3)	0/0	44 \pm 25	210 \pm 120	430 \pm 400
Pd anticholinergic (3)	0/0	16 \pm 1.4	200 \pm 110	146 \pm 14
Pd untreated (3)	0/0	52 \pm 43	260 \pm 80	200 \pm 110
Pd (3)	0/2 000	2 800 \pm 270	15 000 \pm 9 700	380 \pm 60
Pd (2)	0/4 500	15 500 \pm 9 900	19 500 \pm 8 700	1 400 \pm 510
Pd (4)	30/300	16 000 \pm 9 500	15 000 \pm 5 600	830 \pm 400
Pd (1)	60/600	27 000	16 000	800
Pd (7)	75/750	28 000 \pm 21 000	11 500 \pm 7 400	930 \pm 390
Pd (6)	100/1 000	31 000 \pm 22 000	10 000 \pm 6 800	1 900 \pm 1 600
Pd (1)	125/1 250	34 000	10 000	2 500
Pd (1)	150/1 500	25 000	14 000	1 600

6 additional Parkinson patients on other doses of carbidopa/DOPA were analysed (data not shown) and gave results consistent with the above data.

Elmer Sigma 10 Data Station, and identified by co-migration with the authentic standards. Catecholamine values were compared with normal and neurological controls as well as with known literature values (8, 16).

RESULTS

Normal chromatogram

In the normal chromatogram, multiple peaks were present. The compounds of interest eluted in the following order: DA (7.0 min), DOPA (9.5 min) and 5SCD (15.5 min). The compounds recovered with the following percentages: DA (52), DOPA (57), and 5SCD (66). However, results were based on the ratio of peak areas of each compound in the sample, versus the corresponding standards added to the pool. Calculations were corrected for endogenous catecholamines in the pool and internal standard recovery. The values for the normal pool are seen in Table 1. Values from 10 additional out-patient normal subjects and neurological controls were slightly higher, probably reflecting increased activity.

Urine DOPA

The urine excretion of free DOPA in three unmedicated Parkinson patients was similar to controls. DOPA excretion in 3 patients receiving anticholinergics alone was also similar to controls (Table 1). In patients taking DOPA, the urinary excretion of DOPA varied between 0.15 and 0.40%

of the amount ingested. Increased excretion of DOPA with increased dose is seen in Fig. 1A (- carbidopa). In patients taking carbidopa and DOPA in a 1:10 ratio (Fig. 1a, + carbidopa), the amount of urinary excretion of DOPA increased 7-8-fold. The percentage excreted also increased to about 2.5% of that ingested.

Urine DA and DA/DOPA + DA Ratio

In the 3 unmedicated Parkinson patients and the 3 patients taking anticholinergics, the DA/DOPA + DA ratio (0.83-0.92) was similar to controls (0.82-0.95) (Fig. 2). With DOPA therapy, the DA/DOPA + DA ratio was 0.60-0.90 but the DA excretion was always higher than DOPA. With the ingestion of DOPA + carbidopa, the DA/DOPA + DA ratio was lower (Fig. 2). At 75/750 ($N=7$), the amount of DA was always less than DOPA and the ratio was 0.33. About 50-75 mg of Carbidopa gave a 50 percent reduction in the DA/DOPA + DA ratio.

Urine 5-S-Cysteinyldopa

The upper limit of normal of 5SCD excretion in our controls is 200 $\mu\text{g/day}$, with a borderline range of 200-400 $\mu\text{g/day}$. The urinary excretion of 5-S-Cysteinyldopa in 3 unmedicated Parkinson patients was in the normal-to-low borderline range. Three patients who were on anticholinergics alone had normal urinary 5SCD excretion. Among patients who received low-dose DOPA (Fig. 1B, - Carbidopa), 5SCD values were borderline range. In patients taking higher doses, 5SCD was markedly increased.

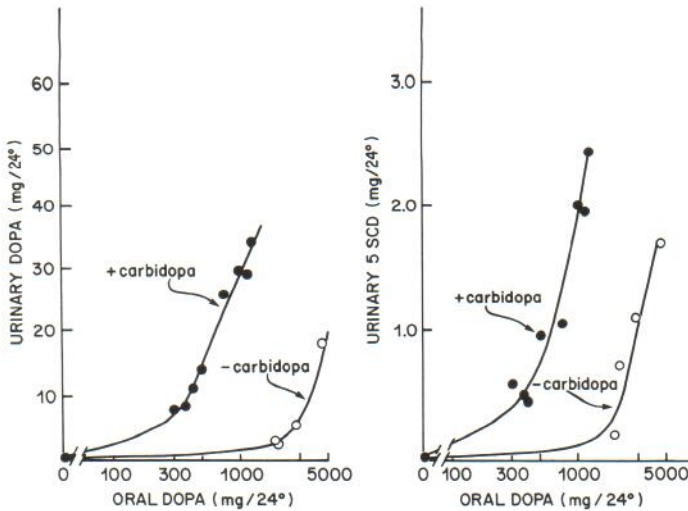


Fig. 1. Urine excretion of DOPA (A) and 5-S-CysteinylDopa (B) following treatment with DOPA with (+) and without (-) carbidopa.

Addition of carbidopa further elevated 5SCD excretion (Fig. 1 B, + carbidopa; Table I).

In 2 patients with metastatic malignant melanoma, 5SCD excretion was 850 and 378 $\mu\text{g}/\text{day}$. Another, without metastasis, had 66 $\mu\text{g}/\text{day}$. In some treated Parkinson patients, 5SCD values were higher than in some melanoma patients (Fig. 3).

DISCUSSION

The catechol metabolism of the melanocyte has been intensively investigated, especially as these cells possess a special pathway for the conversion of DOPA, utilizing the enzyme tyrosinase (17, 18). DOPA and dopamine were initially suggested as possible biological markers of melanocyte activity, as these compounds had elevated levels in some patients with metastatic melanoma (8). More recently, urinary 5SCD, as catechol metabolite of DOPA, has been found to be elevated in a majority of patients with metastatic melanoma (9-12). This compound has also been demonstrated in both human and experimental melanoma cells (18, 19). Tyrosinase oxidizes DOPA to dopaquinone to which addition of cysteine causes 5SCD to form (19). In contrast to DOPA, 5SCD is metabolized by O-methylation rather than decarboxylation (20), and appears to be a better marker for melanocyte activity than DOPA or dopamine.

Urinary 5SCD was essentially normal in 3 unmedicated patients and was normal in 2 patients treated with anticholinergics. This compound was elevated in patients on higher doses of DOPA or

after moderate doses of DOPA and carbidopa which inhibited dopa decarboxylase. The increased 5SCD excretion in Parkinson patients noted here following DOPA therapy parallels and extends the observation previously thought to be limited to melanoma patients (21). In fact, some of our Parkinson patients treated with DOPA and carbidopa had higher 5SCD values than unmedicated patients with malignant melanoma. Although the increased 5SCD

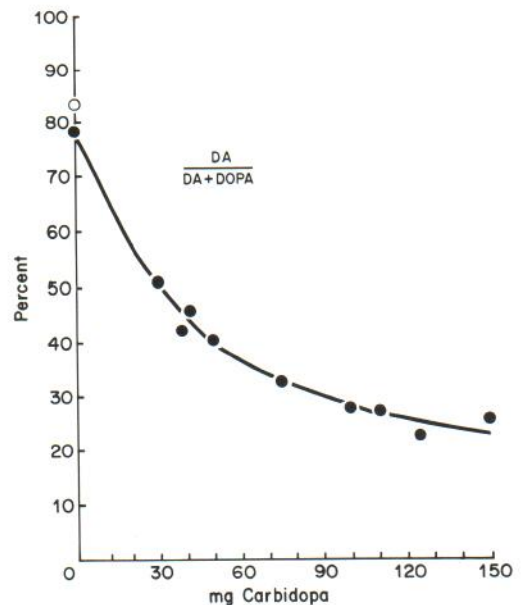


Fig. 2. Decreasing ratio of urinary DA to DOPA + DA with increasing doses of DOPA and carbidopa.

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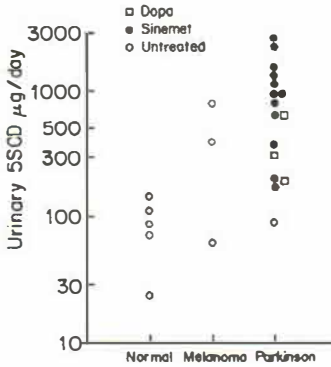


Fig. 3. Urinary 5-S-Cysteinyldopa in controls, patients with malignant melanoma, and Parkinson disease.

appears to reflect a greater substrate availability of DOPA for the melanocyte. 5SCD has recently been noted in sympathetic ganglion cells (22) and may perhaps originate from other neurocrest derivatives.

The further elevation of 5SCD in our patients after dopa decarboxylase inhibition would be consistent with the reported enhancement of DOPA incorporation into subcutaneous melanoma cell lines in the mouse after dopa decarboxylase inhibition (23). We tried to assess the degree of peripheral DOPA decarboxylation in our patients by comparing the amounts of DOPA and dopamine excreted in the urine. The ratio of DA/DOPA + DA was selected because the sum of DOPA and DA accounted for more than 90% of the urinary catechol derivatives in treated Parkinson patients (Fig. 2). This ratio was high in controls and in the untreated Parkinson patients (0.85–0.92). The ratio was somewhat lower (0.60–0.90) in DOPA-treated patients, but DA excretion was always higher than DOPA. In patients treated with both carbidopa and DOPA, the ratio was much lower and urinary DOPA excretion exceeded DA at doses of carbidopa above 75 mg. About 50–75 mg of carbidopa was needed to reduce this ratio by 50% (Fig. 2). This amount of carbidopa was associated with an enhanced 5SCD excretion (Fig. 1), suggesting that increased 5SCD excretion occurred following dopa decarboxylase inhibition.

Our finding of increased 5SCD after DOPA therapy suggests that melanocyte activity in man

can be enhanced by increased substrate availability of DOPA, but does not permit any conclusion about the therapeutic risks of melanoma induction after long-term DOPA usage. Other stimuli such as long-term exposure to ultraviolet radiation which may increase the risk of inducing melanoma, also increases 5SCD production acutely (9, 24). This factor must be seriously considered when measuring urinary 5SCD in a sunny climate. Nevertheless, alternative anti-Parkinson agents such as the direct acting dopamine agonists have been suggested for the melanoma patient with Parkinsonism (6, 7). Since we did not find an increased 5SCD excretion in our Parkinson patients treated with anticholinergics, such an alternative therapy might permit the accurate monitoring of urinary 5SCD as a marker of melanocyte activity in the melanoma patient with Parkinson's disease and perhaps remove a potential therapeutic risk.

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