Acta Dermatovener (Stockholm) 63: 147–182, 1983

SHORT REPORTS

Demonstration of 5,6-Dihydroxyindole-2-Carboxylic Acid, a Melanin Precursor, in Normal Urine

Christer Hansson

Departments of Dermatology and Pharmacology, University of Lund, Lund, Sweden

Received July 24, 1982

Abstract. 5,6-Dihydroxyindole-2-carboxylic acid has been identified in normal human urine. In normal subjects the mean urine concentration was 5.2 ng/ml (range 2.2–8.0 ng/ml).

Key words: Melanin; Urine; Indole derivatives

Metabolism of dopa in the epidermal melanocyte is known to occur mainly by formation of cysteinyldopa compounds when an excess of cysteine or glutathione is present (6). The relatively low content of sulphur usually found in eumelanin indicates, however, that the amounts of thiols available in the melanosomes may be low (4). The finding of an indole glucuronide in melanoma urine is direct evidence of dopa oxidation in tissues with low concentrations of mercaptans (5). Earlier work has shown that methylated derivatives of indoles are present in melanoma urine but in no case has an immediate precursor of eumelanin been demonstrated. This study shows that 5,6-dihydroxyindole-2-carboxylic acid (5,6-DHI-2-C) occurs in normal urine (Fig. 1).

MATERIAL AND METHODS

5,6-Dihydroxyindole-2-carboxylic acid was synthesized *ad modum* Benigni & Minnis (1). Analytical studies were performed by HPLC with fluorometric detection. A column packed with octadecyl silica (Nucleosil C_{18}, 5 μm) and a mobile phase containing phosphoric acid (3 g) in 20% (v/v) aqueous methanol at pH 4.0 were used. Native fluorescence was measured at excitation/emission wavelengths of 325/405 nm.

24-hour urine specimens were collected in plastic bottles containing 50 ml of acetic acid and 1 g of sodium metabisulphite and stored in a refrigerator. 200-ml aliquots of the urine were adsorbed onto alumina (5 g) at pH 8.6 in the presence of EDTA (500 mg) and sodium metabisulphite (50 mg). The samples were stirred for 5 min, centrifuged three times at 4000 rpm and washed with 25 ml of water between each centrifugation. The adsorbed compounds were then eluted with 5 ml of 1 M perchloric acid.

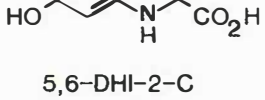
In some experiments the eluate was chromatographed on a semipreparative column (300×8 mm, Microbondapack C_{18}, 10 μm, Waters Ass.) in portions of 600 μl using 5% methanol in water acidified to pH 4.0 with concentrated acetic acid as mobile phase. The peak with the same retention time as synthetic 5,6-DHI-2-C was collected and the pooled fractions were concentrated to 1 ml on a Rotavapor. 100 μl of this concentrated urinary sample was evaporated to near dryness with a stream of nitrogen and 100 μl of butanol was added in three portions with evaporation between each addition to remove remaining water. The sample was then esterified with butanol (1 ml) containing HCl (3 M) for 15 min at 60°C.

The butyl ester of synthetic 5,6-DHI-2-C (1 mg) was also prepared by esterification with 3 M HCl in butanol (1 ml) at 60°C for 15 min.

RESULTS

Chromatographic analysis of urinary samples after elution from alumina showed a distinct peak at the same retention time as that of synthetic 5,6-DHI-2-C (Fig. 2). A mixture of the synthetic 5,6-DHI-2-C and the urinary eluate showed one homogeneous peak at the expected retention time. When the pH and the methanol concentration of the mobile phase were changed, no separation of synthetic 5,6-DHI-2-C and the compound studied occurred. Adsorption chromatography on a Lichrospher silica gel column using heptane : ethyl acetate : methanol (6 : 4 : 0.2) saturated with water as mobile phase further confirmed the presence of 5,6-DHI-2-C in the urinary eluate.

An aliquot of the concentrated urinary sample



5,6-DHI-2-C

Fig. 1.

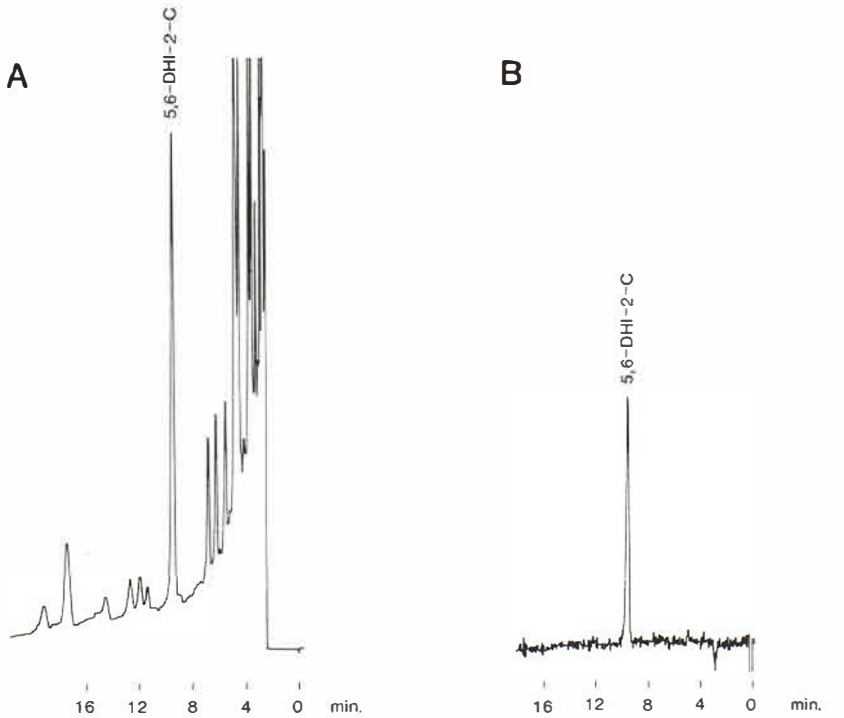


Fig. 2. Chromatograms of normal urine after purification on (*a*) alumina, and (*b*) alumina, followed by semipreparative HPLC. Mobile phase: 3 g H_3PO_4 in 1 litre 20% aqueous methanol at pH 4.00 Column: Nucleosil C_{18} (5 μm, 250×5 mm).

after purification on alumina and on a semipreparative HPLC column was analysed by fluorescence spectroscopy. The compound in the urinary aliquot showed the same fluorescence characteristics as synthetic 5,6-DHI-2-C when analysed in the concentrated mobile phase (λ_{ex} 320 nm; λ_{em} 400 nm).

We now confirmed the presence of 5,6-DHI-2-C in urine also by processing the concentrated sample after purification on a semipreparative column according to the Fisher esterification procedure in butanol.

This experiment showed the disappearance of 5,6-DHI-2-C and simultaneous formation of a compound with the same chromatographic properties as the synthetic butyl ester of 5,6-DHI-2-C.

To exclude the artificial formation of 5,6-DHI-2-C from dopa originally present in urine, large amounts of dopa were added to a urinary aliquot and the urine was subjected to the procedure described. This procedure gave no increase in the concentration of 5,6-DHI-2-C.

In three subjects with normal excretion of 5-S-cysteinyldopa the mean urine 5,6-DHI-2-C concentration was 5.2 ng/ml (range 2.2–8.0 ng/ml). One patient with melanoma metastasis and increased 5-S-cysteinyldopa urine concentration (3 200 ng/ml) showed a normal value of urinary 5,6-DHI-2-C. One patient operated on for primary melanoma showed a normal concentration of urinary 5-S-cysteinyldopa, but the urine concentration of 5,6-DHI-2-C was 21.6 ng/ml.

DISCUSSION

The results presented in this study demonstrate the presence of 5,6-DHI-2-C in normal urine. The chromatographic properties of the urinary compound were extensively studied and found to correspond to the properties of synthetic 5,6-DHI-2-C. The excitation and emission wavelengths of the urinary and the synthetic compounds were found to be identical. Further proof that 5,6-DHI-2-C is present in normal urine was obtained by formation of 5,6-DHI-2-C butyl ester when processing an aliquot of the purified urine with acidic butanol.

5,6-DHI-2-C has been considered to be formed predominantly under acidic conditions (3), but the presence of this compound in normal urine shows that it is formed even at neutral pH. The site of formation of 5,6-DHI-2-C cannot be established from this study, but an earlier observation of meth-

ylated indole derivatives in melanoma urine indicates formation in the melanocyte.

The urinary excretion of 5,6-DHI-2-C did not increase with increasing concentration of 5-S-cysteinyldopa. This finding is similar to that obtained with serum dopa concentrations which were shown to remain at a normal level also in patients with melanoma metastases—except for one patient with an extremely high serum 5-S-cysteinyldopa concentration (2).

Complex mixtures of indole metabolites have been used in the diagnosis of malignant melanoma. Due to the instability of 5,6-DHI-2-C this compound will probably not be suitable for routine analysis of melanoma urine, but investigations on dopa metabolism and pigment formation should in the future also include this new substance.

ACKNOWLEDGEMENTS

This work was supported by grants from the Swedish Cancer Society (project 626-B82-10XA) and the donation funds of the Faculty of Medicine, University of Lund.

REFERENCES

1. Benigni, J. D. & Minnis, R. L.: The syntheses of 5,6-dihydroxyindole and some of its derivatives. J Heterocyclic Chem *2:* 387, 1965.
2. Hansson, C., Edholm, L.-E., Agrup, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: The quantitative determination of 5-S-cysteinyldopa and dopa in normal serum and in serum from patients with malignant melanoma by means of high-pressure liquid chromatography. Clin Chim Acta *88:* 419, 1978.
3. Mason, H. S.: The chemistry of melanin. III. Mechanism of the oxidation of dihydroxyphenylalanine by tyrosinase. J Biol Chem *172:* 83, 1948.
4. Nicolaus, R. A.: Melanins. Hermann, Paris, 1968.
5. Pavel, S., Muskiet, F. A. J., Nagel, G. T., Schwippelova, Z. & Duchon, J.: Identification of two Thormählen-positive compounds from melanotic urine by gas chromatography–mass spectrometry. J Chromatogr *222:* 329, 1981.
6. Rorsman, H., Agrup, G., Hansson, C., Rosengren, A.-M. & Rosengren, E.: Detection of pheomelanins. *In* Pigment Cell, vol. 4 (ed. S. N. Klaus), p. 244. Karger, Basel, 1979.

The Effect of UVB Light on Serum Concentrations of 5-S-Cysteinyldopa

E. Tegner, G. Agrup and E. Rosengren

Departments of Dermatology and Pharmacology, University of Lund, Lund, Sweden

Received May 7, 1982

Abstract. 5-S-Cysteinyldopa concentrations in serum were studied in patients with psoriasis treated with UVB light. An increase in 5-S-cysteinyldopa was found after three treatments. The highest values were noted after 5 weeks' treatment.

Key words: 5-S-Cysteinyldopa; UVB light; Melanocytes; Psoriasis

There is a current trend back to the use of UVB phototherapy for psoriasis (2, 3, 9, 10, 11). Comparative studies have been made concerning the efficacy of phototherapy and photochemotherapy (15). Exposure to sunlight leads to increased urinary excretion of the melanocytic metabolite 5-S-cysteinyldopa (13). In a series of investigations we studied the effect of various treatments with artificial light on 5-S-cysteinyldopa (1, 7, 14). We have monitored the urinary excretion and serum concentrations of 5-S-cysteinyldopa in patients with psoriasis treated with PUVA, and found a marked increase in 5-S-cysteinyldopa after 3 days' treatment, at which time no pigmentation had yet appeared. The highest concentrations in urine and serum were noted after 1 to 3 weeks' treatment (1, 7). Recently we have also studied 5-S-cysteinyldopa concentrations in serum in healthy individuals after exposure to UVA light alone, and found a marked increase after 3 days' irradiation and still higher individual concentrations after 7 to 10 days (14). The aim of the present study was to observe the chemical events in the melanocytes after exposure to artificial UVB light as reflected by changes in serum concentrations of 5-S-cysteinyldopa.

MATERIAL AND METHODS

12 otherwise healthy psoriasis patients were included in the study. All were outpatients. The extent of the skin lesions varied between 10% and 50%, i.e. only moderate psoriasis; patients with more generalized disease are given treatment other than UVB light alone. All 12 patients