Identification of 5-S-Cysteinyldopa by High Performance Liquid Chromatography in Biopsies from Patients with Dysplastic Melanocytic Nevi

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ABSTRACT

The appearance of 5-S-cysteinyldopa, a major product in pheomelanogenesis was examined in affected and unaffected skins from 20 patients with clinical signs of dysplastic melanocytic nevi. Analysis by high performance liquid chromatography and electrochemical detection showed that 20 of the 35 lesions had a pathological formation of 5-S-cysteinyldopa (0.04–28.86 ng/μg acid soluble protein). 5-S-cysteinyldopa was not detected in any of the normal uninvolved skin samples analyzed.

INTRODUCTION

Cysteinyldopas are a group of important intermediates, with 5-SCD2 (Fig. 1) as the major compound, mainly involved in the formation of pheomelanin (1). 5-SCD is formed by all active melanocytes with pheomelanogenesis, and high quantities are present in the melanoma tissue itself; increased levels have been observed in the urine from melanoma patients (2–11). The use of HPLC with electrochemical detection has become the choice for many investigators in detection of 5-SCD due to its high sensitivity and specificity (7–12).

Recent reports have suggested that individuals having dysplastic nevi or DMN are predisposed to develop cutaneous melanoma (13–17). DMN were first called “BK moles” and described as familial linked since they were associated with familial malignant melanoma (13), although these nevi were also observed in cases with nonfamilial melanoma (14). Clinical and histological studies seem to suggest that DMN may be transformed into melanomas. About 22–36% of sporadic malignant melanomas may arise directly from DMN (15), and patients with a history of melanoma tend to have a higher frequency of DMN than do subjects without melanoma (18).

In this study we investigated DMN from a chemical standpoint by measuring the amount of 5-SCD using reverse-phase HPLC and electrochemical detection. Increased 5-SCD was detected in skin lesions of DMN, while skin from the same individual contained no 5-SCD.

MATERIALS AND METHODS

Specimens. Thirty-five biopsies were taken from DMN lesions diagnosed in 20 Caucasians, 14 male and 6 female (obtained from a private dermatological practice in Napa, CA). Biopsies were mainly from lesions on the covered area. The age of the patients ranged from 10–78 years (M = 36); all had fair skin but hair and eye color varied (Table 1). Normal uninvolved skin was also taken from skin adjacent to the lesions for comparison. The tissues were frozen in liquid N2 immediately after dissection and stored at -80°C.

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Clinical Characterization. The lesions were divided into three classes:

1. Normal uninvolved skin was taken from skin adjacent to the lesions for comparison. The tissues were frozen in liquid N2 immediately after dissection and stored at -80°C.

2. No degradation of added 5-SCD to uninvolved skin samples was observed during storage and work-up procedure. The recovery of 5-SCD from PBA-60 gel was 95–102% which is in agreement with that obtained with urine (10) and melanoma cells (12).

3. HPLC Assay. The HPLC apparatus consisted of a Beckman Model 110A solvent pump (Beckman Instruments, Inc., Berkeley, CA), a Rheodyne Model 7125 sample injector (Rheodyne Inc., Cotati, CA), and an LC-3A electrochemical detector (Bioanalytical Systems, West Lafayette, IN) operating at +750 mV versus an Ag/AgCl reference electrode and with a glassy carbon as working electrode. The analytical column was a C-18 LiChrosorb column (10 μm; 250 x 4.6 mm; Alltech Associates, Inc., Deerfield, IL). A mixture of 62.5 mM methanesulfonic acid, 30.6 mM phosphoric acid, and 0.1 mM EDTA in Millipore-filtered water, adjusted to pH 2.9 with 5 N NaOH was used as the mobile phase at a flow rate of 1.5 ml/min. The detection limit for 5-SCD was 2.5–4.0 ng/ml (twice the signal/noise ratio; 100 μl injected). Each sample was analyzed twice.

RESULTS

Table 1 summarizes age, sex, color of hair and eyes, and clinical appearance of the skin lesions at the time of biopsy. The majority (17 samples) of the involved lesions were clinically considered to be mild DMN, 9 were moderate, 4 were between the classes, 3 were other types of melanocytic disorders, and 2 lesions were not recorded.

5-SCD was not detected in any of the 35 uninvolved skin samples analyzed. In contrast 5-SCD ranging from 0.014-28.89 ng/μg soluble protein was detected in biopsies obtained from 13 of the patients (Table 1). The remaining 14 involved skin biopsies showed no 5-SCD. A typical HPLC chromatogram of a positive skin sample is shown in Fig. 2, with a trace of uninvolved skin for comparison. The peak of 5-SCD coeluted with that of our synthetically prepared standard (which had identical structural characteristics by proton nuclear magnetic resonance and mass spectrometry as reported by Agrup et al. (6)).

DISCUSSION

5-SCD of various amounts was detected in biopsies of DMN lesions. No correlation was found between the amounts of 5-
SCD and the degree of clinical appearance. Since 5-SCD was not detected in normal skin of the same individuals, we consider that activated melanocytes are responsible for the production of 5-SCD. The HPLC tracing of the involved skin extracts showed a pattern similar to that of B16 melanoma cells prepared in the same way (12), suggesting possible similarities in the metabolic changes between DMN and melanoma. Most patients in our study with positive 5-SCD identification showed a family history of DMN. Five patients (Table 1, A, D, F, M, and Q) had previously been treated for DMN, and two (Table 1, H and P) of them had family members that had malignant melanoma.

Whether or not DMN represent a premalignant condition has been debated. Clark et al. (13), Elder et al. (14), and Kraemer et al. (15) reported that lesions clinically identified as DMN lesions later changed to show clinically and histologically features of malignant melanoma. Nordlund et al. (18) recently reported in a demographic study from Australia that fair-skinned patients with sporadic melanoma had a higher tendency to have DMN lesions than did a normal control group. Of the patients with melanoma 34% had one or more DMN, compared to 7% in the control group. This is higher than that found in an earlier study done on a similar population in Napa, CA (4.9%, confirmed histologically) where we obtained our biopsies (20). Nordlund et al. (18) also showed that no differences were seen in the melanomas between patients with or without DMN. Younger subjects (under 50 years) had significantly more DMN per individual than did older subjects. The sex of the individual did not have any effect on the appearance of DMN.

It is premature to conclude that DMN containing 5-SCD really will be transformed into melanoma. A possibility is that 5-SCD is formed by melanocytes metabolically activated by mechanisms other than that of malignancy. Agrup et al. (5) reported that fair-skinned people normally showed higher urine excretion of 5-SCD than did dark-skinned subjects and that increased levels of 5-SCD were observed in individuals that had been exposed to sunlight (21). The excretion of 5-SCD in the urine of healthy human subjects regardless of hair color and age was reported not to be significantly different from that of patients with primary melanoma (3, 5). Moreover normal values in the urine were seen even in patients with metastases (5). In addition we showed recently that chemicals such as the antioxidants induced the formation of 5-SCD in cultured melanocytes (12, 22, 23). Scientific technology is now available for quantitative assay of 5-SCD in the diagnosis of melanoma and other skin conditions clinically related. Careful investigation with large numbers of tissue samples chosen from a variety of conditions is expected to clarify whether DMN may (or may not) be a biomarker of a higher risk in developing malignant melanoma.

REFERENCES


5-SCD, DMN, AND CUTANEOUS MELANOMA


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