Sulfatase Activity of Keratinizing Tissues in X-Linked Ichthyosis

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Summary

Arylsulfatases A and B were measured in the stratum corneum of four normal controls and two individuals with sex-linked ichthyosis. For arylsulfatase A, the mean Δ optical density/hr/mg protein value was 1.6 for controls and 2.0 for patients, whereas for arylsulfatase B values of 1.5 for controls and 1.4 for patients were observed.

Assay of arylsulfatase C in the callus of four normal controls showed a mean Δ optical density/hr/100 mg callus of 0.63, whereas no or trace activity was detected in callus from four patients with x-linked ichthyosis.

The assay of steroid sulfatase is best for studying microsomal sulfatase activity. Table 1 shows the activity of this enzyme in nails, callus, and hair bulbs from controls and patients with x-linked ichthyosis. No steroid sulfatase could be demonstrated in patients with x-linked ichthyosis. The values in normal controls and obligate heterozygotes are compared in Table 2. The mean value of the two groups is statistically different with $P \leq 0.05$ using the Student *t* test.

Speculation

Analysis of keratinizing tissues for steroid sulfatase activity can be used for the definitive diagnosis of x-linked ichthyosis and may be of value in identifying heterozygotes. Analysis of these tissues may also be useful in studying a number of metabolic disturbances because other enzymes are also present.

Patients with x-linked ichthyosis have been shown to have a deficiency of the microsomal enzymes, arylsulfatase C, and steroid sulfatase in their cultured fibroblasts and epidermal cells (2, 3, 9, 10). Arylsulfatase A and B, which are lysosomal enzymes, are present in normal amounts in the disease. The enzyme defect is not present in other conditions such as ichthyosis vulgaris, lamellar ichthyosis, epidermolytic hyperkeratosis, and psoriasis. Arylsulfatase C has been studied in hair and appeared to be decreased (5) in sex-linked ichthyosis.

This report describes the assay of arylsulfatases A, B, and C and steroid sulfatase in nail, stratum corneum, and hair bulbs allowing the demonstration of enzyme deficiency without using invasive techniques.

MATERIALS AND METHODS

The patients with sex-linked ichthyosis were referred to the Dermatology Genetics Clinic at the Massachusetts General Hospital, and the obligate heterozygotes were their mothers (other male relatives were affected) or daughters. Stratum corneum was shaved from the heel, weighed, and used immediately or stored for up to 10 days at -70°C which resulted in no loss of activity. The tissue was ground in 20 mM Tris buffer, pH 7.0, in a conical glass homogenizer. Nails were clipped from the fingers whereas hair was plucked from the scalp using needle holders. The bulbs

were clipped off, and the anagen ones were isolated and counted using a dissecting microscope. The nails were rinsed with Tris buffer and dropped into the reaction mixture, whereas the hairs were ground in the assay buffer.

Steroid sulfatase was assayed as previously described with tritium-labeled dehydroepiandrosterone sulfate using 0.4 ml of a 50 mg/ml callus suspension, 30 mg of nail in 0.4 ml of buffer, or 20 hair bulbs homogenized in 0.4 ml of buffer (3). Aryl-sulfatase A and B were done on a 30,000 $\times$ g supernatant of homogenized callus which had a protein content of about 0.5 to 1.0 mg/ml. The reaction mixtures consisted of 0.4 ml *p*-nitrocatechol sulfate (2 mg/ml) in 1 M sodium acetate at pH 5.0 (for A) or 6.0 (for B) plus 0.2 ml of enzyme extract (8). After incubation at 37°C for 1 hr, 0.4 ml of 6 N sodium hydroxide was added, and the solution was read against a blank at 420 nm. Arylsulfatase C was done using 0.72 ml of a stratum corneum suspension (100 mg/ml) in 0.25 M Tris-acetate buffer, pH 8.2, and 0.08 ml of 100 mM *p*-nitrophenyl sulfate (8). After incubation at 37°C for 1 hr with shaking, the suspension was clarified by centrifugation, 0.4 ml of 6 N sodium hydroxide was added, and the solution read at 420 nm.

Protein content of the supernatant solutions was measured by the Bio-Rad method. The water content of the callus samples was quite constant at $30 \pm 5\%$ as determined by the loss in weight of a piece of callus after drying at 80°C .

RESULTS

Arylsulfatases A and B were measured in duplicate in the stratum corneum of four normal controls and two individuals with sex-linked ichthyosis. For arylsulfatase A, the mean Δ optical density/hr/mg protein value was 1.6 (range, 1.3 to 1.8) for controls and 2.0 (range, 1.6 to 2.3) for patients, whereas for arylsulfatase B values of 1.5 (range, 1.2 to 1.7) for controls and 1.4 (range, 1.2 to 1.5) for patients were observed.

Assay of arylsulfatase C in the callus of four normal controls showed a mean Δ optical density/hr/100 mg callus of 0.63 (range, 0.40 to 0.78) whereas no trace activity was detected in callus from four patients with x-linked ichthyosis. The assay of arylsulfatase C is somewhat less sensitive than those of A and B, but absence of any activity in patients with x-linked ichthyosis indicates there is no interference by arylsulfatase A and B of the arylsulfatase C assay.

The assay of steroid sulfatase is quite sensitive because of the use of a radioactive substrate and is best for studying microsomal sulfatase activity. Table 1 shows the activity of this enzyme in nails, callus, and hair bulbs from controls and patients with x-linked ichthyosis. No steroid sulfatase could be demonstrated in patients with x-linked ichthyosis. Two patients with ichthyosis vulgaris showed values of 720 and 801 pmoles dehydroepiandrosterone per hr for callus steroid sulfatase activity. The values in normal controls and obligate heterozygotes are compared in Table 2. The mean value of the two groups is statistically different with $P \leq 0.05$ using the Student *t* test. Thus, the heterozygotes show a trend of having lower values of steroid sulfatase activity.

Table 1. Steroid sulfatase activity of nail, hair, and stratum corneum¹

	pmoles dehydroepiandrosterone/hr	
	Normal	x-linked
Nail (30 mg)	110 ± 20 (4) ²	0 (4)
Hair (20 bulbs)	30 ± 5 (4)	0 (4)
Callus (20 mg)	560 ± 127 (9)	0 (9)

¹ The data give the mean value of the pmoles dehydroepiandrosterone produced per hr.

² Numbers in parentheses, number patients.

Table 2. Steroid sulfatase activity for controls and obligate heterozygotes of x-linked ichthyosis¹

pmoles dehydroepiandrosterone/hr	
Controls	
1	502
2	734
3	530
4	428
5	694
6	670
7	614
8	520
9	352
Mean ± S.D.	560 ± 127
Heterozygotes	
1 ^m	262
2 ^d	242
3 ^m	84
4 ^m	366
5 ^d	510
6 ^d	390
7 ^d	236
8 ^d	28
Mean ± S.D.	265 ± 159

¹ The assays were done with 0.4 ml of a 50 mg/ml suspension of callus, and the values are the average of duplicate determinations.

² m, mother of an affected individual; d, daughter of an affected individual.

DISCUSSION

A number of disorders have been associated with sulfatase deficiencies (1), and the most recently reported is x-linked ichthyosis (10), which shows absent or very low levels of arylsulfatase C and steroid sulfatase. It is thought that these are different enzymes (1) which suggests that the defect may involve control of microsomal sulfatase synthesis. The association of ichthyosis with

arylsulfatase C deficiency was reported earlier in a group of patients with mixed sulfatase deficiency (6), but the connection was not recognized at the time. As mentioned by Shapiro (10) only patients with the multiple deficiencies that included arylsulfatase C had ichthyosis. However, it has not been possible to ascertain from the published reports whether the clinical features were typical of x-linked ichthyosis.

Histochemical techniques (2, 5) can be used diagnostically to detect arylsulfatase C activity in the skin, but analysis of steroid sulfatase in stratum corneum offers a sensitive, noninvasive test for the diagnosis of x-linked ichthyosis. Although the disease can ordinarily be recognized accurately by its clinical features, the assay is of help in problem cases and those with atypical features. Furthermore, there appears to be a trend for the heterozygotes to have lower values of sulfatase activity relative to the mean. As more data are collected from different series of cases a more accurate interpretation of the test will be possible.

The analysis of enzyme activity in cornified tissue might be of value in studying other metabolic disturbances. Analysis of stratum corneum for urocanic acid and histidase activity, for example, has been used for the diagnosis of histidinemia (4) and hair roots have been used in G6PD deficiency (7).

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