URINARY MUCOPOLYSACCHARIDES IN ACHEIROPODIA

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Urinary mucopolysaccharides from three patients with acheiropodia were qualitatively and quantitatively analysed by agar gel electrophoresis coupled with enzymatic degradation. Although no abnormal pattern was characterized, eventual metabolic dysfunction detected only in bone/cartilage tissues could not be ruled out.

INTRODUCTION

Acheiropodia is a special type of congenital malformation in wich the phenotype reveals characteristic symmetric " amputations " of the four limbs (Freire-Maia et al. 1958, Toledo and Saldanha 1969). It results from multiple aplasias and hypoplasias caused by a rearrangement of the embryonic inductors, inherited as an autosomal recessive trait. This entity seems to be limited to the Brazilian territory (Toledo et al. 1972, Freire-Maia 1975, Freire-Maia et al. 1975). It has been recently reported that chondroitin sulphate A (CSA) is deficient in limb cartilages of the chick embryos with a special type of inherited nanomelic dwarfism that could somewhat resemble acheiropodia (Matthews 1967). Thus, it was decided to analyse the mucopolysaccharides (MPS) of patients with this disease.

MATERIAL AND METHODS

The three cases studied with acheiropodia belonged to a sibship of 11 members (Toledo and Saldanha 1969, Toledo et al. 1972). The affected patients: V.M., J.V.M. and P.M. were 20, 15 and 8 years of age, respectively.

Urinary MPS (Berry-Spinanger spot test and Dorfman-Steiness test) were within the normal values. Urine without preservation collected during 24 h was kept refrigerated under 4°C. Extraction and identification of the urinary MPS were made as follows. Urinary MPS from 40 normal individuals and three patients with acheiropodia were extracted from urine samples with cetyltrimethylamnonium bromide (Meyer et al. 1958), after dilution with half volume of distilled water. The MPS were identified and quantitated by a combination of agarose gel electrophoresis and enzymatic degradation, as previously described (Dietrich and Dietrich 1972, Dietrich et al. 1973). Standards of CSA and chondroitin sulphate C (CSC) were purchased from Miles Laboratories, Inc. (Elkhart, Indiana, USA). Heparitin sulphate (HS) was prepared as previously reported (Dietrich et al. 1971).

RESULTS

Table 1 shows the electrophoretic mobility of the two main MPS fractions, namely CSA and CSC (fraction I) and HS (fraction II), extracted from the urine of the three cases with acheiropodia and from a pool of 40 normal subjects. These data were also compared with those of Murata et al. (1970). No significant differences in the relative electrophoretic mobility of the MPS were noticed between the two groups. Table 2 shows the amount of disaccharides formed by the enzymatic degradation of the urinary MPS with chondroitinase AC. No one of the cases showed increased or decreased amounts of unsatured, non-sulphated disaccharides. Unsaturated 4-sulphate (from CSA) and the unsaturated 6-sulphated (from CSC) disaccharides of the affected patients were formed in similar amounts when compared with those of normal urines.

Table 1. *Some properties of urinary MPS from patients with acheiropodia*

Patients	Urinary MPS (mg/l)	Electrophoretic migration		Susceptibility to chondroitinase AC		Susceptibility to chondroitinase AC and heparitinases	
		Fraction 1	Fraction 2	Fraction 1	Fraction 2	Fraction 1	Fraction 2
V.M.	6	4.1	3.4	+	—	+	+
J.V.M.	12	4.0	3.3	+	—	+	+
P.M.	17	3.9	3.4	+	—	+	+
Norm. subjects	10 (5-20)	4.1 (3.9-4.2)	3.4 (3.3-3.5)	+	—	+	+

Table 2. *Disaccharides formed by the action of chondroitinase AC upon urinary MPS from patients with acheiropodia*

Patients	Chondroitinase AC disaccharides (%)		
	Di4S	Di6S	DiOS
V.M.	43	55	2
J.V.M.	44	53	3
P.M.	27	73	1
Normal subjects	37 (32-42)	62 (57-67)	<5
Normal subjects (Murata et al. 1970)	36 (32-42)	54 (48-60)	7 (3-9)

DISCUSSION

Many metabolic disturbances of the MPS have been recently found in bone dysplasias (BD) (McKusick 1972, Spranger 1972). Furthermore, urinary increase of CSA and/or CSC was observed in a series of patients with BD (Philippart and Sugarman 1969, Spranger et al. 1971, Benson et al. 1972, Baberick et al. 1974, Schimke et al. 1974). A special type of BD was recently reported in which CSC was excreted within normal levels, but with a significantly low sulphate content (Mourão et al. 1973, Toledo et al. 1973). In chick embryos, a specific type of recessive inherited nanomelic dwarfism was correlated with a deficiency of CSA in limb cartilages (Matthews 1967). In the last two conditions it was assumed that a mechanism of synthesis is defective.

Urinary MPS in acheiropodia were studied in an attempt to detect some inherited metabolic disturbances possibly related to the present bone anomalies. However, no qualitative or quantitative abnormality was observed in them. This evidence supports the hypothesis that a structural anomaly of the CSA and/or CSC is absent in acheiropodia. In spite of this, further studies of cartilage specific enzymes (Lash and Marzullo 1966) should be necessary to rule out an eventual metabolic disturbance detectable only in limb cartilage/bone tissues. So far, no biochemical anomaly has been detected in this entity (Toledo et al. 1972).

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