SYNTHESIS AND DEGRADATION OF COLLAGEN BY SKIN FIBROBLASTS FROM CONTROLS AND FROM PATIENTS WITH OSTEOGENESIS IMPERFECTA

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Received 16 March 1979

1. Introduction

Osteogenesis imperfecta (OI) is a heritable disorder affecting bone. Typically such patients have fragile bones and may also have blue sclerae, dentinogenesis imperfecta, hearing loss, thin skin, joint hypermobility, herniae and mitral valve prolapse [1]. The severity of the disorder varies considerably in different patients, and both dominant and recessive forms are known. This heterogeneity indicates that there must be differences in the molecular basis which thus far have escaped definition. In cases with more generalized affection of connective tissues, it has been proposed that OI is caused by defects in the structure or metabolism of type I collagen, the principal structural protein of bone, dentin and many other tissues. This concept is supported by studies demonstrating that fibroblasts from certain affected individuals synthesize collagen types I and III in an abnormally low I/III ratio [2,3] and that skin collagen in some patients is present in a decreased I/III ratio [4].

A low ratio of I/III collagen may reflect either reduced type I collagen synthesis or enhanced intracellular collagen degradation. To test this, we evaluated collagen production and intracellular collagen degradation in 9 normal individuals of varying ages and in 16 patients with OI, including strain CRL-1262 [2]. Since only OI strain CRL-1262 produced less than normal amounts of collagen but had normal degradation, we conclude that at least one form of the disease is associated with a decrease in collagen synthesis.

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2. Materials and methods

2.1. *Skin fibroblasts*

All cells were obtained from American Type Culture Collection, Rockville, MD. The fibroblasts from 9 normal individuals included CRL-: 1106, 1121, 1141, 1222, 1187, 1220, 1224, 1120 and 1221, representing donors from fetal to 84 years. Fibroblasts from 16 patients with OI were studied including CRL-: 1298, 1286, 1251, 1315, 1293, 1288, 1267, 1107, 1287, 1280, 1129, 1248, 1247, 1294, 1381 and 1262, representing donors from fetal to 64 years. The subcultivation numbers of all cells were between 4 and 8. The cells were cultured and subcultured according to routine techniques.

2.2. *Labelling of cells*

Cells at early confluence were preincubated with medium supplemented with ascorbic acid (60 μg/ml) for 16 h, rinsed twice with phosphate-buffered saline and incubated with 50 μCi [^{14}C]proline (285 mCi/mmol) in 5 ml fresh medium containing 10% dialyzed fetal calf serum, 2 mM glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml), ascorbic acid (60 μg/ml) and β-aminopropionitrile (50 μg/ml). After 8 h, medium and cell layer were combined, sonicated and processed as described below.

2.3. *Quantitation of collagen production*

The ratio of collagen to total protein production was assessed by both the hydroxyproline content and susceptibility to collagenase. Hydroxyproline was determined by a modified [5] chemical procedure [6] or by ion-exchange chromatography on Dowex 50 [7] and the amount of collagen per total protein synthesized was calculated [8]. Collagenase-sensitive material was determined using purified bacterial collagenase [9].

2.4. *Quantitation of intracellular degradation of newly synthesized collagen [10,11]*

One half of the medium plus cell layer was lyophilized and hydrolyzed (6 N HCl, 110°C, 24 h) and used to measure the total amount of [^{14}C]-hydroxyproline present in the culture. The other half was dialyzed against a 4-fold volume of 0.5 M acetic acid for 48 h at 4°C. The dialyzate was lyophilized and hydrolyzed, and dialyzable [^{14}C]hydroxyproline was quantitated making appropriate corrections for the volume in the dialysis bag.

3. Results

The quantity of collagen produced by all but one strain of OI fibroblasts was similar to that of the control cells. However, OI cell strain CRL-1262 produced only 60% of collagen (table 1). In a separate experiment we found that the apparent reduction of collagen synthesis in CRL-1262 cells was not due to production of underhydroxylated collagen. When collagenous proteins in medium and cell layer were measured with bacterial collagenase, collagen production was found to be decreased in medium and cell layer by ~40%, while the distribution of collagen between medium and cell layer was normal (table 2).

We tested whether the decrease in overall collagen production found in CRL-1262 was due to increased intracellular degradation. Collagen degradation by control fibroblasts averaged 30 ± 4% (mean ± SEM, range 25–37%), was independent of donor age (fig.1) and similar to that found in human fetal lung fibroblasts [11]. Degradation by cells from all patients with OI was in the same range (29 ± 6%, fig.2); degradation by CRL 1262 fibroblasts (fig.2, open circles) measured 34% and 31% (duplicates from two separate experiments).

Table 2
Production and distribution of collagen in medium and cell layer of OI (CRL-1262) and control (CRL-1121) fibroblasts

		Collagen per total protein (%)	
		Hydroxyproline method [7,8]	Collagenase method [9]
Medium	OI	11.8	13.9
	control	24.1	23.6
Cell layer	OI	1.7	2.0
	control	3.3	3.4
Medium and cell layer	CI	7.8	9.0
	control	15.2	14.3

Table 1
Production of protein-bound hydroxyproline [5,6] by OI and control skin fibroblasts

Cell strains	Protein-bound hydroxyproline (dpm)	
	Total incorporated radioactivity (dpm) mean ± SEM	(range)
9 controls	0.15 ± 0.02	(0.13 –0.20)
15 osteogenesis imperfecta all strains except CRL-1262	0.14 ± 0.01	(0.12 –0.17)
1 osteogenesis imperfecta strain CRL-1262	0.86	(0.079–0.088)[a]

[a] Mean and range of 4 separate measurements

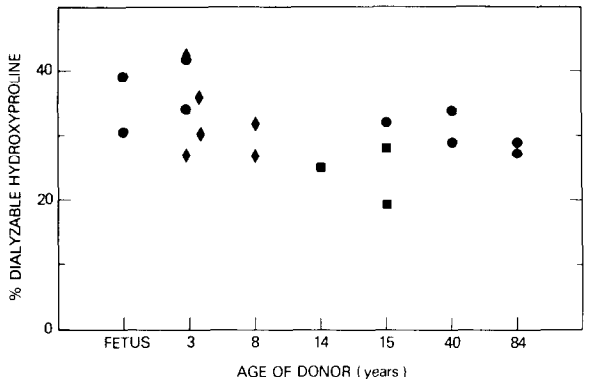

Fig.1. Intracellular collagen degradation by skin fibroblasts derived from 8 normal donors of different ages, expressed as % dialyzable hydroxyproline per total [^{14}C]hydroxyproline. The symbols (●, ■, ♦) represent values from 3 different experiments.

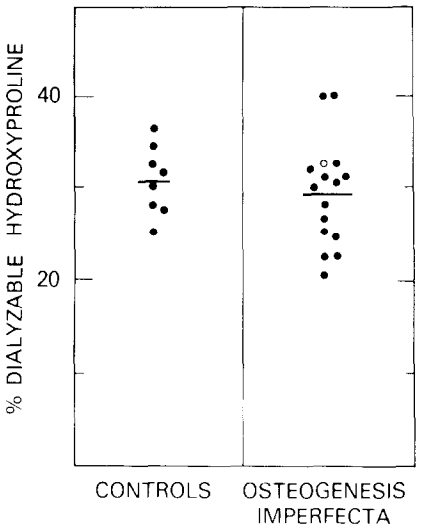

Fig.2. Intracellular collagen degradation by cells from controls and OI patients. Each point represents the mean value for a given cell strain, each horizontal bar the mean value for a group. CRL-1262 is characterized by (○).

4. Discussion

Collagen synthesis was decreased in one (CRL-1262) of 16 cell strains derived from patients with OI. We obtained similar results both by measuring peptide-bound hydroxyproline and collagenase-digestible protein.

Intracellular collagen degradation was examined for the first time in human skin fibroblasts and was shown to be independent of donor age. Therefore, degradation by CRL-1262 cells, derived from a fetus, could be compared. As in all other OI cells, collagen degradation was normal. Since CRL-1262 produced only half the normal amount of collagen, this suggested that the underlying defect in this case was not due to a post-translational process but rather to an abnormality at the transcriptional or translational level(s).

Since type I collagen accounts for 85–90% of the total collagen in cultured fibroblasts, the 40% deficit in strain CRL-1262 was likely due to a decrease in type I collagen synthesis which is reflected by the decreased I/III collagen ratio [2]. The tissue fragility in this OI patient [12] suggested that tissue levels of collagen were also reduced in vivo. Membranes ruptured prematurely after weeks 30–32 of gestation, and there was a traumatic breech presentation with avulsion of head and one foot. All connective tissues were most fragile. Histologically, there was not only a decrease in collagen in endochondral and membranous bones but also in teeth, corneae, sclerae, ligaments and skin. Interestingly, the papillary layer of the dermis was relatively well preserved whereas the reticular layer was very thin. From immunofluorescent studies [13] it is known that the papillary dermis is rich in type III collagen whereas most of the reticular dermis is composed of type I collagen (these conclusions have recently been contested [14]). Although biochemical studies on skin were not performed we believe that the discrepancy between the involvement of papillary and reticular layers is the pathological correlate of reduced type I collagen synthesis.

Thus, CRL-1262 cells stood out from a series of 16 patients with OI in that they synthesized less collagen. Very recently, David Rowe of the University of Connecticut has made two similar observations (personal communication), a finding which may prove to be characteristic of a novel subclass of osteogenesis imperfecta.

Acknowledgement

We wish to thank Huldrych Bingesser for his skillful assistance and Drs Robert Bienkowski and Richard Gitzelmann for helpful discussions and suggestions.

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