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Altered ratio of collagen chains in bone of a patient with non-lethal osteogenesis imperfecta

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Bone from a patient with osteogenesis imperfecta contained type III collagen which was absent in control bone. The ratio of $\alpha 1(I)/\alpha 2(I)$ in type I collagen of patient's bone was increased (2.9 vs. 2.3 $\pm$ 0.2 in controls) and the ratio of dimers $\beta 11/\beta 12/\beta 22$ was altered due to the increased $\beta 22$ content. No abnormality was observed in collagen from the patient's skin. The altered composition of collagen in bone, but the normal composition in skin suggests that the disease in the patient is due to impaired regulation of the synthesis of collagens in bone, rather than by a mutation in one of the two type I collagen genes. Unlike in skin, all the type III collagen in patient's bone was pepsin-soluble indicating an inability of the bone to incorporate type III collagen into mature highly cross-linked extracellular matrix.

Introduction

Osteogenesis imperfecta (OI) is a group of inherited conditions whose main feature is excessive fragility of bones, which may be accompanied by a variety of other connective tissue abnormalities [1]. Recent data have demonstrated that most forms of OI are caused by mutations in one of the two structural genes for type I procollagen (for review see Refs. 2–4). Currently it is not completely clear how the molecular defect is translated into the tissue abnormality and then into the clinical phenotype. In addition, linkage analysis has shown that in several families OI is not caused by mutation in type I collagen genes [5,6]. In order to better define the biochemical alterations underlying OI we have studied collagens from bone and skin of a patient with OI type III [1]. The data suggest that an impaired regulation of collagen synthesis in bone plays an important role in the etiopathogenesis of OI. In addition, analysis of the composition of pepsin-soluble and pepsin-insoluble fractions of collagens from bone and skin of the patient and controls indicates an inability of bone to fully utilize type III collagen as a structural component, since it was not incorporated into cross-linked matrix.

Abbreviations: OI, osteogenesis imperfecta; HAc, acetic acid; CNBr, cyanogen bromide.

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Materials and Methods

Case report

The patient was a 1 year and 3 month old male at the time of biopsy. After birth bow-shaped tibiae and femurs were observed; a turicephal head and rhisomelic shortening of femurs were noted as well. At the age of 1 month a fracture of the humeral diaphysis occurred. Upon X-ray examination, healed fractures of both femurs were noticeable. The patient had hip motions limited to 45 degrees. The patient currently presents short stature, shorter leg to trunk ratio and a larger forehead than normal.

Methods

Specimens of the patient's bone from the iliac crest and specimens of skin were obtained by biopsy during surgery. Control tissue specimens were obtained by

autopsy from 20 children, aged 3–14 years, with no evidence of connective tissue diseases. Histology did not reveal any significant differences in the patient's bone (data not shown). Bone fragments were carefully separated from any soft tissues, demineralized by dialysis against three changes of 0.5 M EDTA, 0.05 M Tris-HCl (pH 7.5), 10 mM phenylmethansulphonyl fluoride, homogenized and then extracted with 0.5 M acetic acid (HAc) for 48 h. After centrifugation, the insoluble residue was digested with pepsin (100 μg/ml in 0.5 M HAc) for 48 h and centrifuged. The supernatant (pepsin-soluble fraction) and insoluble residue (pepsin-insoluble fraction) were collected and freeze dried. Similar amounts of collagen were solubilized from control and the patient's bone (between 40 and 60% of the total) confirming earlier observations [7,8]. Pieces of skin were defatted, finely minced with scissors, thoroughly washed with 50 mM EDTA, 10 mM phenylmethansulfonyl fluoride and then with water. Samples were homogenized and pepsin-soluble and pepsin-insoluble fractions were obtained as described for bone samples.

Results

Fig. 1 shows results of unreduced electrophoresis of pepsin-soluble collagens extracted from bone and skin of the patient and control child. Normal skin is known to contain type I and type III collagens, whereas normal bone contain only type I collagen [7,9,10]. Pepsin-soluble collagen from skin of the patient had normal composition and similarly to control it revealed under unreduced electrophoresis: molecules $[\alpha 1(III)]_3$ of type III collagen; and $\alpha 1(I)$, $\alpha 2(I)$ chains, $\beta 11$, $\beta 12$ dimers and γ component of type I collagen (Fig. 1). Pepsin-soluble collagen from control bone did not contain molecules $[\alpha 1(III)]_3$. Pepsin-soluble collagen from bone of the patient differed from control by the presence of molecules $[\alpha 1(III)]_3$ of type III collagen (Fig. 1). Molecules $[\alpha 1(III)]_3$ were not observed after reduction of disulphide bonds by 2-mercaptoethanol (data not shown). The intensity of $\beta 22$ band was increased in the patient (Fig. 1). Densitometry of electrophoregrams showed that the ratio $\beta 11/\beta 12/\beta 22$ in the patient was

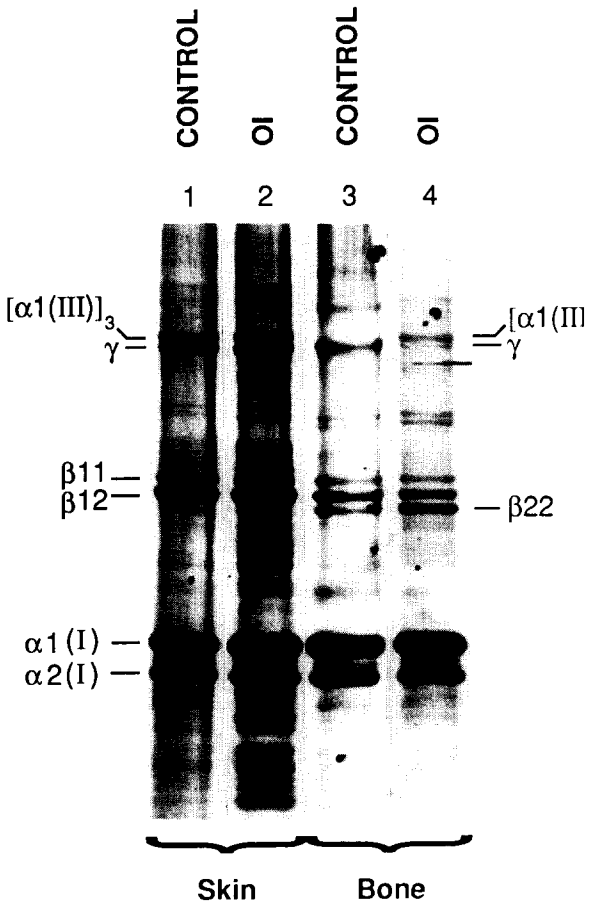

Fig. 1. Unreduced SDS polyacrylamide gel electrophoresis of collagens extracted by limited pepsin digestion from bone and skin of the patient with osteogenesis imperfecta and control child. Lanes 1, 3 – skin and bone of control child. Lanes 2, 4 – skin and bone of the patient.

1.0/1.7/1.7 vs. 1.0/1.7/1.0 in controls (Table I). Evaluation of the densitometric profiles also revealed an increased ratio of chains $\alpha 1(I)/\alpha 2(I)$ in type I collagen from bone of the patient (2.9 vs. 2.3 ± 0.2 in controls). In contrast to bone, the $\alpha 1(I)/\alpha 2(I)$ ratio in the skin from the patient did not differ from controls (Table I).

Fig. 2 shows an electrophoregram of pepsin-soluble and pepsin-insoluble collagens subjected to cyanogen bromide (CNBr) digestion [11]. Pepsin-soluble collagen from bone of the patient contained CNBr-peptides of

TABLE I

Comparison of some characteristics of bone and skin collagens from the patient with osteogenesis imperfecta and from control children

Origin of specimen	Percentage of type III collagen				$\alpha 1(I)/\alpha 2(I)$		$\beta 11/\beta 12/\beta 22$
	skin		bone		skin	bone	bone
	pepsin soluble fraction	insoluble fraction	pepsin soluble fraction	insoluble fraction			
Patient	26 ± 2.1	25 ± 3.1	60 ± 5.3	0	2.3 ± 0.1	2.9 ± 0.2	1.0/1.7/1.7
Controls	22 ± 4.3	22 ± 1.5	0	0	2.3 ± 0.2	2.3 ± 0.2	1.0/1.7/1.0

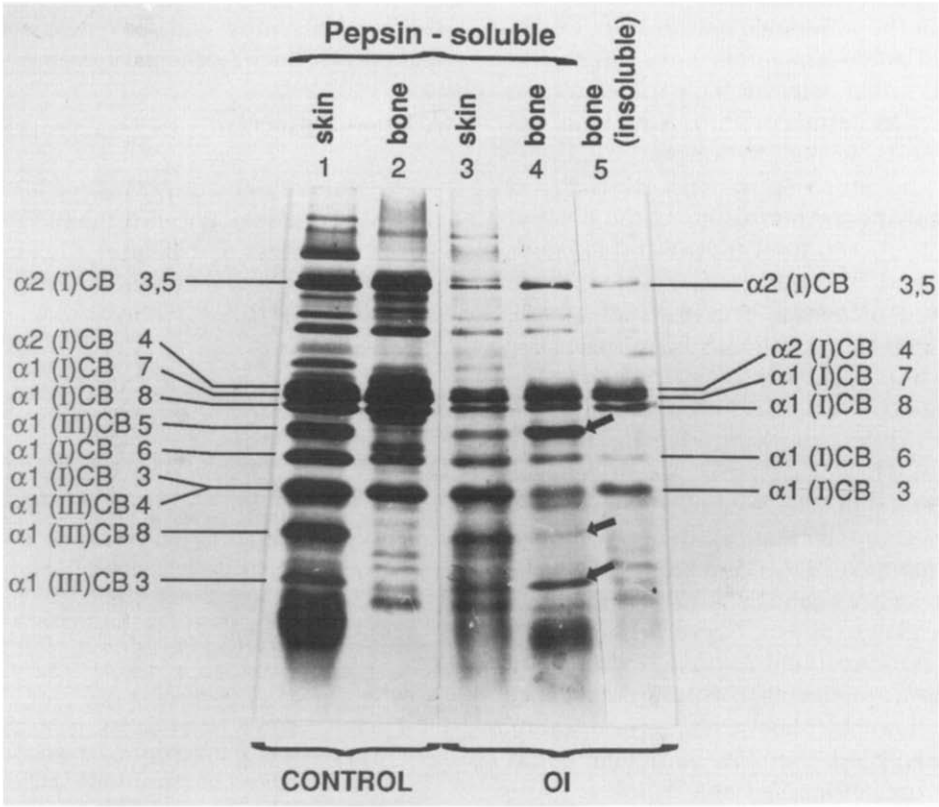

Fig. 2. Unreduced SDS polyacrylamide gel electrophoresis of collagens cleaved by cyanogen bromide. Lanes 1, 2 – pepsin soluble fractions from control skin and bone, respectively. Lanes 3, 4 – pepsin soluble fractions from patient's skin and bone, respectively. Lane 5 – insoluble collagen from OI bone. Arrows in the line of pepsin-soluble collagen from bone of patient show CNBr-peptides α1(III)CB5, α1(III)CB8 and α1(III)CB3 of type III collagen.

type III collagen in addition to CNBr-peptides of type I collagen (Fig. 2). Evaluation of type III collagen content using densitometric areas of CNBr-peptides α1(III)CB5 (marker peptide for type III collagen) and α1(I)CB8 (marker peptide for type I collagen), which are not known to be involved with cross-linking [12], showed that type III collagen represents about 60% of total pepsin-soluble collagen in OI bone (Table I). However, type III collagen CNBr-peptides were not detectable in pepsin-insoluble collagen of patient's bone and in both pepsin-soluble and pepsin-insoluble collagens from controls bone (Fig. 2, Table I). In contrast to bone, skin from both the patient and controls contained type III collagen in a similar proportion to type I collagen in both pepsin-soluble and pepsin-insoluble fractions (Table I). The type III collagen content of the patient's skin was not altered significantly when compared to controls (Table I).

Discussion

The bone from the patient with OI described in this communication contains a considerable amount of type III collagen which is absent in normal bone [7,9,10]. Previously, detectable quantities of type III collagen in bone have been shown in several other patients with OI [7,10,13,14]. However, the distribution of type III collagen between different fractions of collagen from OI bone has not been investigated. Here we have shown that type III collagen was present only in the pepsin-soluble fraction of collagen from bone of patient with OI, but was absent in the pepsin-insoluble fraction. This indicates that type III collagen in the patient's bone has not been incorporated into the mature highly cross-linked collagen matrix. In contrast to bone, the skin of the patient and the controls contained type III collagen in the same proportions in both pepsin-soluble and pepsin-insoluble fractions. Thus the inability of bone to fully utilize type III collagen is caused not by primary defect in type III collagen, but by the absence in bone of appropriate mechanisms to incorporate type III collagen into mature matrix. These data suggest that one or several of the enzymes operating posttranslational modifications necessary for cross-linking of collagens, is specific for type III collagen and this enzyme(s) is absent in bone, but present in skin. Interestingly, the presence of a considerable amount of type III collagen did not markedly effect an amount of inorganic material in bone of the patient. The amount of inorganic material

was calculated from the difference between dry weight of bone before and after demineralization. No significant difference was noted between the patient and the control group. In every instance, mineral material accounted for some 60% of bone dry weight.

In addition to the presence of type III collagen, which is uncharacteristic for normal bone, the bone of the patient with OI differed from controls by composition of type I collagen. The ratio $\alpha 1(I)/\alpha 2(I)$ in bone from the patient was increased. The presence of $\beta 22$ dimers was quite high in the patient's bone suggesting either an altered fibril structure or altered cross-linking. In contrast to bone, type I collagen in the skin of the patient did not differ from controls. Nor was the proportion of type III collagen to type I collagen increased in patient's skin. The normal composition of collagen in skin of the patient as both as normal electrophoretical patterns of CNBr-peptides of type I collagen (Fig. 2) evidence against a structural mutation in one of the two genes for type I procollagen as an explanation of alterations found in bone. Existence of cases of OI which are unlinked to type I procollagen genes have been shown by genetic analysis of several families [5,6]. We suggest that the molecular defect causing OI in the patient studied here is located in a mechanism regulating bone-specific synthesis of collagens. It is possible that the presence of type III collagen in bone impairs osteogenesis and therefore mutations which derepress synthesis of type III collagen in bone cause some cases of OI. It should be mentioned that we did not find any type III collagen in bone from several other patients with non-lethal OI. This indicates that the disorder does not represent a single biochemical entity and may be associated by fundamentally distinct biochemical events.

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