Calcif. Tiss. Res. 17, 75—79 (1974)

Bone Collagen Aggregation Abnormalities in Osteogenesis Imperfecta

Steven L. Teitelbaum, William J. Kraft, Robert Lang and Louis V. Avioli

Department of Pathology and Laboratory Medicine
Department of Medicine, Division of Endocrinology, The Jewish Hospital of St. Louis, Washington University School of Medicine

Received May 17, accepted July 14, 1974

Scanning electron microscopy of bone collagen of three patients with osteogenesis imperfecta congenita demonstrates the failure of thin collagen fibers to aggregate extracellularly into the distinct and separately discernible large collagen fiber bundles of normal bone. These organizational alterations may in part explain the changes in physical properties which result in the frequent fractures characterizing this disease.

Key words: Bone — Collagen — Osteogenesis Imperfecta.

Introduction

Osteogenesis imperfecta, a heritable disease of connective tissue usually transmitted as an autosomal dominant, becomes clinically manifest by a predisposition towards multiple bone fractures. Clinical findings also include lax, hypermobile joints, blue sclerae, thin translucent skin, dentinogenesis imperfecta and deafness. It is not unusual for these patients to experience more than one hundred fractures, ultimately resulting in severe bone deformities.

The disease manifests itself in two general clinical patterns. Osteogenesis imperfecta congenita affects the newborn, while osteogenesis imperfecta tarda appears after birth and carries a more favorable prognosis. Although the pathogenesis of this disease is unknown, most investigators invoke alterations of bone turnover or abnormalities of collagen metabolism. Histometric [5, 18] and biochemical [7, 10] studies suggest increased resorptive activity accompanied by normal states of bone formation, resulting in decreased total bone mass.

Evidence of collagen abnormalities in osteogenesis imperfecta is controversial. Brown (1973), studying fibroblast cultures of patients with this disease, reports both decreased hydroxyproline production and collagen cross-linkage, thereby proposing qualitative and quantitative abnormalities of collagen production. Conversely, Scriver (personal communication), examining log phase or confluent cell growth phase of skin fibroblasts from osteogenesis imperfecta patients, notes normal hydroxyproline/proline and hydroxylysine/lysine ratios. On the basis of these data he assumes that hydroxylation of collagen within the cell and ultimate extrusion of the tropocollagen molecule are normal. Additional evidence affirming abnormalities of collagen metabolism include reports of elevated bone aspartic

For reprints: Steven L. Teitelbaum, M.D., The Jewish Hospital of St. Louis, 216 S. Kingshighway, St. Louis, Missouri 63110, USA.

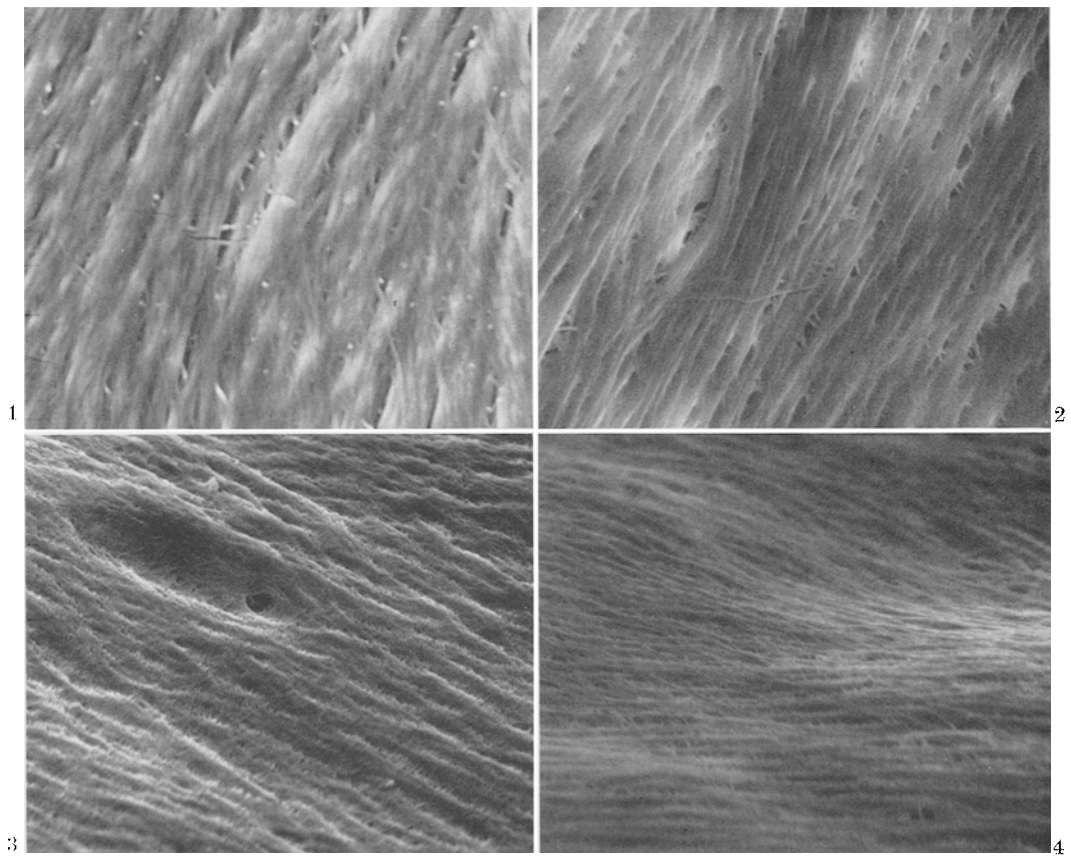

Fig. 1. Control osteoid. Note thick interweaving fiber bundles, × 2200

Fig. 2. Osteoid of patient OI-A. Although some fiber aggregation is present the mean fiber width is approximately one fourth normal, × 2200

Fig. 3. Osteoid of patient OI-B. Fiber bundles are thin and porous, × 2200

Fig. 4. Osteoid of patient OI-C. This osteoid exhibits the most severe aggregation abnormalities. The mean fiber width is approximately one-fifth normal, × 2200

and glutamic acid [12], abnormal collagen cross-linkage [6], and decreased rates of collagen synthesis [6, 11].

Ultrastructural studies of bone collagen of patients with osteogenesis imperfecta are few. Using transmission electron microscopy, Doty and Mathews (1971) and Riley and Brown (1971) were unable to demonstrate alterations of fiber diameter or periodicity. Therefore, while biochemical evidence alludes to abnormal bone collagen in osteogenesis imperfecta, standard ultrastructural investigations have not been fruitful.

Materials and Methods

We obtained iliac crest bone from three Caucasian females with osteogenesis imperfecta aged ten (OI-A), ten (OI-B) and eleven (OI-C) years. Two of these patients are identical

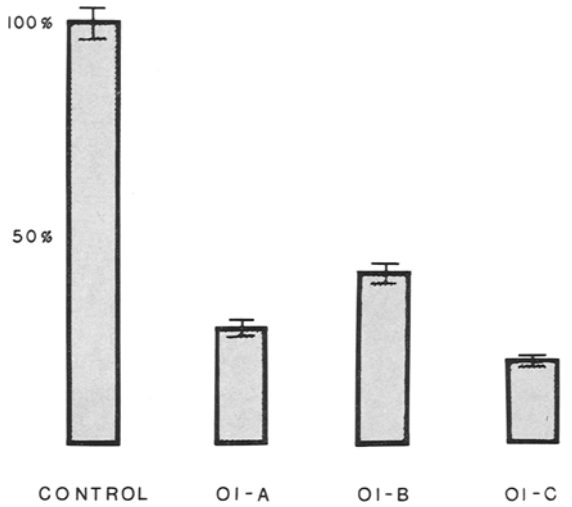

Fig. 5. Fiber width of osteoid tissue obtained from a normal control population (control) and 3 patients (OI-A, OI-B, OI-C) with osteogenesis imperfecta. The data for the osteogenesis imperfecta subjects expressed as percentage of control reflect the mean $\pm$ S.E.M. of 50 individual readings in each instance. For each patient, the deviation from control is significant at $p < 0.001$ according to the students "t" test

twins. All three patients are severely deformed, each having sustained at least 30 fractures. We also examined iliac crest bone from three age and sex matched controls undergoing reconstructive surgery.

Following fixation in 70% ethanol the bone was bathed in 2.5% trypsin for 48 h at room temperature, after which residual marrow was removed with a water jet. The specimens were then coated with chrome in a vacuum evaporator and studied in a Cambridge scanning electron microscope. The widths of identically magnified osteoid fibers of each affected patient were measured and expressed relative to the mean width of 50 randomly selected control fibers.

Results

Thin osteoid fibers of all three patients with osteogenesis imperfecta congenita fail to aggregate into the clearly discernible and ordered thick fiber bundles of normal bone described by Boyde (1972) (Figs. 1—4). The osteoid of the patients with osteogenesis imperfecta appears as sheets of roughly parallel, thin fibers exhibiting defective bundle formation. The mean osteoid fiber width of each patient with osteogenesis imperfecta is significantly abnormal. Relative to the mean width of fifty control fibers expressed as 100 $\pm$3.50% (SEM), the mean osteoid fiber widths of OI-A, OI-B, and OI-C respectively are 25.8$\pm$1.52% (SEM), 40.4$\pm$1.56% (SEM), and 20.2$\pm$0.896% (SEM, Fig. 5). In addition, the fibers OI-B (Fig. 3) appear loosely arranged within the bundle. Although the collagen of this patient's bone assumes a roughly lamellar pattern, many of the thin fibers are randomly oriented, resulting in large interfibrillar spaces and "porous" appearing osteoid.

Discussion

This study demonstrates that the architecture of osteoid in osteogenesis imperfecta is abnormal. The collagen fibers in bone fail to aggregate or pack extracellularly in a normal pattern but are instead organized in the form of thin filaments or loosely compacted bundles. These abnormalities may in part explain the changes in physical properties of bone which result in frequent fractures characterizing osteogenesis imperfecta.

As the factors responsible for the aggregation of collagen fibrils into fibers are virtually unknown [8], the biochemical derangement(s) responsible for the aberrant morphology of bone collagen in osteogenesis imperfecta remain to be defined. Since the fibers, although abnormally aggregated, may be of considerable diameters, the defect would appear to be beyond the enzymatic events which lead to the intramolecular maturation of the tropocollagen molecule [4, 9, 13, 14, 17). The associated extraosseous clinical manifestations of this disease suggest these collagen aberrations may not be unique to bone. Our results are consistent with the concept that the defect in osteogenesis in this disease results primarily from an inherited failure of maturation or differentiation of fetal collagen which normally attends the aging process [16].

Acknowledgement. This study has been supported in part by GRS Grant ÷ 115–123 and Health Science Advancement Award 5 SO4 RR 06115-05.

References

1. Boyde, A.: Scanning electron microscope studies of bone. In: The biochemistry and physiology of bone, vol. 1, 2nd ed. (Bourne, G. H., ed.), p. 259–310. New York: Academic Press 1972
2. Brown, D. M.: Collagen metabolism in fibroblasts from patients with osteogenesis imperfecta (abst.). In: Clinical aspects of metabolic bone disease (Frame, B., Parfitt, E. M., Duncan, H., eds.), p. 303–307. Amsterdam: Excerpta Medica. International Congress Series No. 270 (1973)
3. Doty, S. B., Mathews, R. S.: Electron microscopic and histochemical investigation of osteogenesis imperfecta tarda. Clin. Orthop. **80**, 191–201 (1971)
4. Eyre, D. R., Glimcher, M. J.: Reducible cross-links in hydroxylysine-deficient collagens of a heritable disorder of connective tissue. Proc. nat. Acad. Sci. (Wash.) **69**, 2594–2598 (1972)
5. Falvo, K. A., Bullough, P. G.: Osteogenesis imperfecta: a histometric analysis. J. Bone Jnt Surg. A **55**, 275–286 (1973)
6. Francis, M. J. O., Smith, R., MacMillan, D. C.: Calcitonin in osteogenesis imperfecta. Clin. Sci. **44**, 429–438 (1973)
7. Gardner, B., Wallach, S., Gray, H., Baker, R. K.: Bone collagenase in assessment of the clinical course of metabolic bone disease. Surg. Forum **22**, 435–437 (1971)
8. Glimcher, M. J., Krane, S. M.: The organization and structure of bone and the mechanism of calcification. In: Treatise on collagen, vol. 2, part B (B. S. Gould, ed.), p. 67–251. New York: Academic Press 1968
9. Grant, M. E., Prockop, D. J.: The biosynthesis of collagen. New Engl. J. Med. **286**, 194–199 (1972)
10. Langness, U., Behnke, A.: Collagen metabolites in plasma and urine in osteogenesis imperfecta. Metabolism **20**, 456–463 (1971)
11. Martin, G. R., Layman, D. L., Narayanan, A. S., Nigra, T. P., Siegel, R. C.: Collagen synthesis by cultured human fibroblasts. Israel J. med. Sci. **7**, 455–456 (1971)
12. Niemann, K. N. W.: Amino acid composition of bone collagen in osteogenesis imperfecta. J. Bone Jnt Surg. A **51**, 804 (Abst.) (1969)

13. Pinnell, S. R., Krane, S. M., Kenzora, J. E., Glimcher, M. J.: A heritable disorder of connective tissue. New Engl. J. Med. **286**, 1913–1920 (1972)
14. Pinnell, S. R., Martin, G. R.: The cross-linking of collagen and elastin: enzymatic conversion of lysine in peptide linkage to α-amino-adipic-δ-semialdehyde (allysine) by an extract from bone. Proc. nat. Acad. Sci. (Wash.) **61**, 708–716 (1968)
15. Riley, F. C., Brown, D. M.: Morphological and biochemical studies in osteogenesis imperfecta. J. Lab. clin. Med. **78**, 1000 (Abst.) (1971)
16. Robins, S. P., Shimkomaki, M., Bailey, A. J.: The chemistry cross-links. Age-related changes in the reducible components of intact bovine collagen fibers. Biochem. J. **131**, 771–880 (1973)
17. Tanzer, M. L.: Cross-linking of collagen. Science **180**, 561–566 (1973)
18. Villanueva, A. R., Frost, H. M.: Bone formation in human osteogenesis imperfecta, measured by tetracycline bone labeling. Acta orthop. scand. **41**, 531–538 (1970)