Calcif Tissue Int (1991) 49:248–250

Calcified Tissue International

Changes in Apatite Crystal Size in Bones of Patients with Osteogenesis Imperfecta

U. Vetter, E. D. Eanes, J. B. Kopp, J. D. Termine, and P. Gehron Robey

NIH, National Institute of Dental Research, BRB, Bldg. 30, Room 106, Bethesda, Maryland 20892, USA

Received July 2, 1990, and in revised form December 10, 1990

Summary. Apatite crystal size in compact bone of children (age <11 years) and adolescents (age >12 years) with osteogenesis imperfecta (OI) was analyzed by X-ray diffraction. Eight type I, 4 type II, 11 type III, and 14 type IV OI patients were studied along with 9 controls. The crystal size was most significantly reduced in type II patients, all of whom had died at birth. Crystal size was also diminished in both children and adolescents with types III and IV, whereas with type I OI, crystal size was reduced in children only, returning to normal in adolescence. There was a trend toward increased bone crystal size with age in both OI patients and controls.

Key words: Osteogenesis imperfecta – Hydroxyapatite crystal size – Mineral content.

Osteogenesis imperfecta (OI) is a rare disease of the connective tissues characterized by bone fragility, osteopenia, progressive skeletal deformities, and often severe growth retardation. Blue sclerae, dental and cardiac abnormalities, joint laxity, and maturity onset deafness can be additional manifestations of this clinically variable disease [1].

Sillence et al. [2] have identified four major subgroups of OI patients. Type I and IV OI patients show an autosomal dominant mode of inheritance and a mild or moderate form of the disease; type II leads to perinatal death; type III is a severe form. Both types II and III OI are genetically heterogeneous and are most often the result of sporadic new mutations [3]. OI most likely results from a variety of deletions, insertions, and point mutations in the genes coding for type I collagen, which have been identified in an increasing number of patients [4].

Although OI is a disease characterized by increased bone fragility, little is known about the mineral phase of bone in this disease. We therefore studied changes in apatite crystal size in patients with different forms of OI in childhood and adolescence.

Materials and Methods

Patients

Bone specimens of the femoral diaphysis from controls aged 5, 8, 10, 14, 28, and 30 years, two premature infants of the 32nd and 35th week of gestation, and a newborn, all of whom had died of nonskeletal diseases, and from 4 patients with type II OI were all obtained during autopsy. Bone specimens of 8 patients with type I, 11 patients with type III, and 14 patients with type IV OI were all obtained during intramedullary rodding.

The phenotype of the type I patients was typical to that previously described [2] and exhibited a clinically mild disease with one to two fractures per year and no significant skeletal deformities or growth retardation. The type II patients had died at birth, showing severe skeletal deformities due to innumerable intrauterine fractures. Type III patients suffered from 10–30 fractures per year and developed severely disabling skeletal deformities and extreme skeletal dwarfism. Type IV patients suffered from about 5–10 fractures per year, growth retardation, and skeletal deformities.

Preparation of Samples

The cortical bone was cleaned of adherent soft tissue, ground to a fine powder in a Spex mill under liquid nitrogen, and lyophilized.

X-ray Diffraction

Powder X-ray diffraction scans of bone apatite, the principal mineral phase, were done on a Rigaku X-ray diffractometer (Danvers, MA) equipped with a graphite monochromater tuned to CuKα radiation (λ = 0.154 nm). Only the 002 and 310 apatite peak profiles were examined. Inadequate intensity or uncorrectable interference from neighboring peaks prevented quantitative evaluation of the rest of the apatite diffraction pattern (Fig. 1A, B). The breadths of the 002 profiles from all bone specimens (37 OI and 9 controls) were measured to obtain information on the size and/or internal perfection of the crystals along their length or c-axis, the direction parallel to the collagen fibrils. The 310 peak breadths from 17 OI and four control bones were also used to obtain similar data perpendicular to the c-axis (i.e., width or thickness). A continuous scan mode was employed to record these peaks with all data collected on a strip chart recorder [5]. The 002 and 310 peak profiles from each sample were recorded a minimum of four times each at an angular velocity of 0.125° 2θ/minute using a time constant of 1θ seconds. The scan ranges for the 002 and 310 peaks were 24.5–27° 2θ and 37.5–42° 2θ, respectively. The angular width or breadth of the 002 diffraction peak (Fig. 1B) was measured at one-half the height of maximum intensity above background. The sample width (β) was obtained from the experimentally observed width (B) using the relation $\beta = (B^2 - b^2)^{1/2}$ [6], where b is the angular width from instrumental broadening effects. A well-crystallized synthetic hydroxyapatite sample of negligible intrinsic broadening was used to determine b. The angular width of the 310 diffraction peak was estimated by measuring the distance between the angular position of the peak maximum and the position at which the high angle side was one-half this height and multiplying this distance by 2. This procedure minimizes neighboring 212 peak interference.

As peak broadening is inversely related to both crystal size and internal lattice perfection (i.e., the broader the peak, the smaller and/or more strained the crystals) [6], the reciprocal of the β values ($1/\beta$: also known as sample crystallinity) was used in order to equate the experimental data more directly to these crystal parameters.

Offprint requests to: U. Vetter

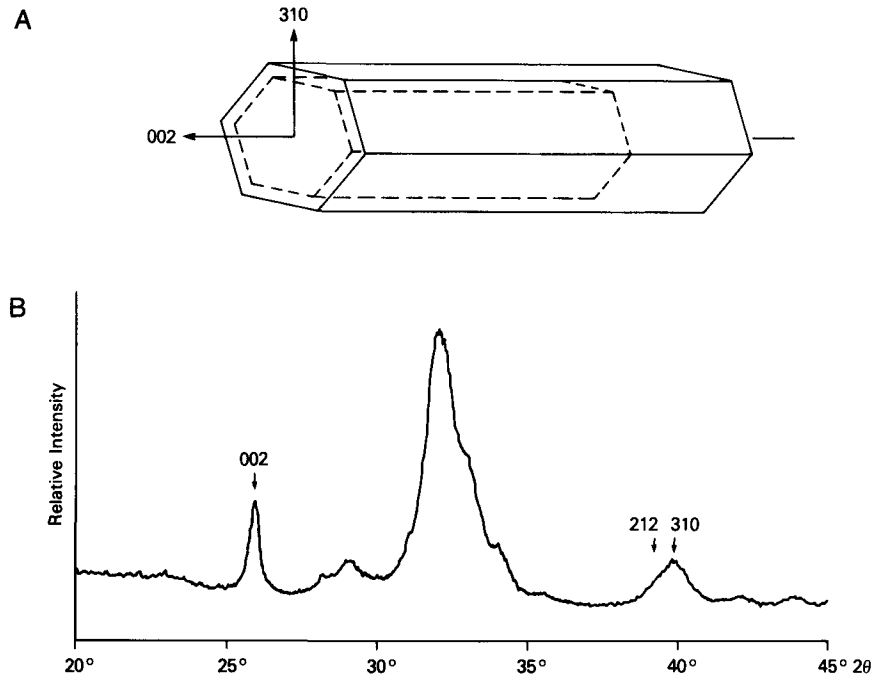

Fig. 1. (**A**) Schematic representation of an apatite crystal showing the relation of the 310 and 002 diffraction vectors to the crystal shape. The dashed lines represent the crystal in the young OI bone and the solid lines represent a mature crystal of normal bone. (**B**) X-ray diffractogram of mature bone showing the 002 and 310 reflections used in the study and the neighboring 212 reflection. The region of highest intensity (30–35° 2θ) consists of four unresolved peaks with unmeasurable overlapping breadths.

Calculation of Crystal Size

In a bovine study [5] of normal and OI bone, transmission electron microscopy data suggested that changes in crystallinity were due to size rather than strain effects. Assuming a similar situation exists for human OI mineral, i.e., strain changes were minimal, then the Scherrer equation [6], $D = \lambda/\beta\cos\theta$, can be used to directly compare changes in crystal size among the bones examined. In this equation, D(nm) is the mean crystallite size, λ is the X-ray wavelength employed, and θ is the Bragg (reflectance) angle expressed in radians. Because the experimental βs were measured in degrees, not radians, these values were divided by 57.3, the radian-degree conversion factor, before applying the above equation for D.

Statistics

For statistical treatment of the data, the Student's *t* tests and linear regression analyses were performed [8].

Results

Comparison of 310 and 002 Crystallinity Data

Regression analysis of the 310 and 002 crystallinity (1/β) data on the 17 OI patients and 4 controls from which both these values were experimentally obtained showed a positive and significant correlation ($r = 0.81$; $P < 0.001$) (Fig. 2). The slope of the regression line (0.17) indicated that the crystallinity changes are anisotropic during crystal growth, i.e., the changes in width/thickness (310) are only 17% as large on average compared with corresponding length (002) changes. Nearly identical results were obtained using only the data from the 17 OI patients (slope = 0.16, r = 0.80). Tables 1 and 2 summarize the crystallinity (1/β) measurements from the 002 and 310 diffraction peaks, respectively. As the crystallinity changes are considerably larger for length than for width/thickness, it was possible to group the length (002) data (Table 1) by age as well as by OI type.

Comparison of 002 Crystallinity and Crystal Size in OI and Normal Bone

Crystallinity along the (002) direction was significantly reduced in bone of type II patients compared with fetal/neonatal controls and in type I, III, and IV children with OI (Table 1). During adolescence, crystal size of type III and IV patients remained significantly reduced, whereas that of type I patients did not differ significantly from age-matched controls. There was also a trend toward increased crystallinity with age in both controls and OI patients (Table 1). With the possible exception of OI type II, the reduced crystallinity changes along the 310 (width/thickness) direction for all OI types were too small to establish statistical significance.

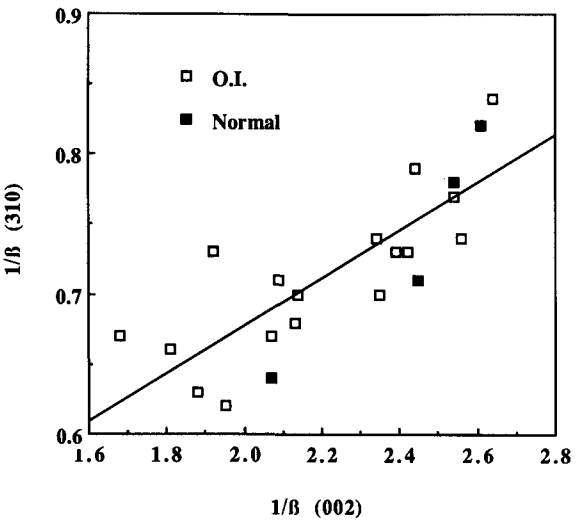

Fig. 2. Plot of 310 versus 002 crystallinity values of 4 control and 17 OI bones. Regression analysis reveals a significant and positive correlation ($r = 0.81$; $P < 0.001$).

Discussion

Bone apatite crystal size was previously determined in two

Table 1. Bone mineral crystallinity (1/β) (1) values (002 or c axis direction) in normal and osteogenesis imperfecta subjects

Type	Fetal/Newborn	Childhood (2–11 years)	Adolescence (≥12 years)
Normal	2.23 ± 0.20 (20.2 ± 1.8 nm) n = 3	2.53 ± 0.11 (22.9 ± 1.0 nm) n = 3 (7.7 ± 2.5 yrs)	2.80 ± 0.25 (25.3 ± 2.3 nm) n = 3 (24 ± 8.7 yrs)
I	—	2.27 ± 0.16[a] (20.6 ± 1.4 nm) n = 5 (9.6 ± 1.7 yrs)	2.54 ± 0.17 (23.0 ± 1.5 nm) n = 3 (14.7 ± 0.6 yrs)
II	1.73 ± 0.07[b] (15.7 ± 0.6 nm) n = 4	—	—
III	—	2.02 ± 0.20[c] (18.3 ± 1.8 nm) n = 7 (7.3 ± 2.1 yrs)	2.33 ± 0.14[a] (21.1 ± 1.3 nm) n = 4 (13.8 ± 2.9 yrs)
IV	—	2.23 ± 0.19[b] (20.2 ± 1.7 nm) n = 9 (4.8 ± 2.6 yrs)	2.34 ± 0.20[a] (21.2 ± 1.8 nm) n = 5 (15.8 ± 3.6 yrs)

[a] Student's t test of difference between OI and means (m ± 1 SD) significant at [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$
The corresponding crystal sizes (nm), calculated according to Scherrer's equation [6], are shown in ( )

Table 2. Bone mineral crystallinity (1/β) values (310 or width/thickness direction) in normal and osteogenesis imperfecta subjects

Type	1/β (310)[a]
Normal	0.74 ± 0.08, n = 4
I	0.77 ± 0.08, n = 3
II	0.66 ± 0.01, n = 2[b]
III	0.70 ± 0.05, n = 4
IV	0.71 ± 0.05, n = 8

[a] Not enough samples for separation of means by age
[b] Student's t test of difference between OI II and normal means significant at $P < 0.2$; all other differences between normal and OI means not significant

models of bovine osteogenesis imperfecta (BOI) [5]. Newborn calves with BOI-Australia and BOI-Texas both showed a decreased apatite crystal size compared with healthy siblings. The changes were isotropic as the decrease in crystallinity was nearly the same for length (002) and width/thickness (310). Crystallinity changes in human OI were anisotropic, showing the most pronounced changes in length (002). The differences between these two situations may be due to species differences in mineralization or a different pathogenesis of bovine and human OI [7].

Changes of crystallinity of human OI bone have not been systematically studied. A previous study only reports that crystal size measured by X-ray diffraction was generally reduced in 17 patients [9]. Our study demonstrates that crystallinity (002) changes correlate with both the clinical phenotype and the clinical course in different forms of OI. For reasons given above under Materials and Methods, we assume that these changes of crystallinity are due to changes in the size of the crystals rather than to strain effects, i.e., imperfections in the crystals. In the type II patients who had died at birth and the severely affected type III patients, crystal size was smallest, whereas mildly affected type I patients showed an almost normal crystal size during adolescence. The reported changes of crystal size in OI bone might reflect an increased bone turnover or a slower crystal growth. Although morphological studies have suggested an increase in bone turnover in OI [10], studies on collagen metabolism have not provided correlative evidence for this assumption [11]. Because crystal growth is tightly associated with collagen fibrils in bone [12], it is fair to speculate that collagen defects in the severely affected patients that lead to disturbed fibril formation [4] also retard crystal growth. Smaller collagen fibrils were indeed demonstrated in an electron microscopic study of the bones of the same OI type II and III patients [13]. However, type I OI patients, whose collagen secretion is thought to be reduced but not qualitatively altered [14], also show a reduced crystal size during childhood. Interestingly, apatite crystal size increased with age in OI bone, just as it did in normal bone. Whether the declining fracture rate in adolescent OI patients is directly related to these observed crystal growth changes remains to be established.

Although it is presently difficult to directly evaluate the consequences of the reported mineral changes on the stability of OI bone, our study presents an initial glimpse into the pathophysiology of mineral deposition in OI bone.

Acknowledgments. This study was supported in part by grants from the Deutsche Forschungsgemeinschaft, D-5300 Bonn-Bad-Godesberg, and the Bundesministerium fuer Forschung und Technologie D-53000 Bonn, FRG.

References

1. McKusick VA (1972) Osteogenesis imperfecta. In: Heritable disorders of the connective tissue. CV Mosby, St. Louis, p 390
2. Sillence DO, Senn AS, Danks DM (1979) Genetic heterogeneity in osteogenesis imperfecta. J Med Genet 16:101–116
3. Thompson EM, Young ID, Hall CM, Pembrey ME (1987) Recurrence risk and prognosis in severe sporadic osteogenesis imperfecta. J Med Genet 24:390–405
4. Byers P (1988) Osteogenesis imperfecta: an update. Growth, Genet Horm 4:1–5
5. Fisher LW, Eanes ED, Denholm LJ, Heywood BR, Termine JD (1987) Two bovine models of osteogenesis imperfecta exhibit decreased apatite crystal size. Calcif Tissue Int 40:282–285
6. Klug HP, Alexander LE (1962) X-ray diffraction procedures. John Wiley, New York, p 491
7. Termine JD, Belcourt AB, Conn KM (1981) Mineral and collagen-binding proteins of fetal calf bone. J Biol Chem 256:10403
8. Green JR, Margerison P (1978) Statistical treatment of experimental data. Elsevier, Amsterdam
9. Volkov MV, Paulova MN, Poliakov AN, Zatzepin ST, Nefedieva NN (1971) Roentgenographic analysis of ultrastructure of basic substance of the bone tissue in osteogenesis. Ortop Travmat Protezi (Moscow) 32:37–42
10. Albright JB, Albright JA, Crelin ES (1975) Osteogenesis imperfecta tarda: the morphology of rib biopsies. Clin Orthop Rel Res 108:204–213
11. Smith R, Francis KJO, Houghton GR (1983) The brittle bone syndrome. Butterworth, London
12. Weiner S, Traub W (1989) Crystal size and organization in bone. Connect Tissue Res 21:259–265
13. Stoess H, Freisinger P (1990) The collagen fibrils of the osteoid in osteogenesis imperfecta—morphometric analysis of the fibril diameter (abstract) vol IV. Int Conf on Osteogenesis Imperfecta, Pavia, Italy
14. Rowe DW, Shapiro JR, Poirier M, Schlesinger S (1985) Diminished type I collagen synthesis and reduced al(I) collagen in RNA in cultured fibroblasts from patients with dominantly inherited (type I) osteogenesis imperfecta. J Clin Invest 71:689–697