Ultrastructural Features of the Osteoid of Patients with Fibrogenesis Imperfecta Ossium

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Abstract

The osteoid of a patient with Fibrogenesis Imperfecta Ossium is described. Three iliac crest biopsies were taken; firstly before treatment, secondly after calcitriol therapy and finally after successful treatment with melphalan and prednisolone. In the pretreatment biopsy the osteoid was greatly enlarged, showed complete absence of the birefringence characteristic of oriented collagen fibers, and at ultrastructural level was shown to be composed of abnormal collagen fibrils. The fibrils were often curved and were extremely variable in thickness. Calcification within the osteoid took the form of calcospherites and spread of calcification from these to collagen fibrils was greatly delayed. In the second biopsy two aspects of osteoid ultrastructure were noted; some samples resembled the first biopsy, but others had a different organization. The osteoid of these samples had two regions: an inner region containing abnormal collagen fibrils and an outer region composed of moderately electron-dense amorphous material. The osteoblasts associated with this region were clearly highly biosynthetically active. The third biopsy, after treatment with Melphalan and prednisolone, showed a reversion to more normal bone ultrastructure with uniform, oriented collagen fibrils and prompt mineralization resulting in narrow osteoid seams. Remnants of the original abnormal osteoid were present in the narrow space as calcified debris. Reasons for the success of this therapeutic regime are unclear; however, some speculation is made as to the possible roles of the cytotoxic drug and the glucocorticoid in the regression of this condition.

Key Words: Bone—Fibrogenesis Imperfecta Ossium—Ultrastructure—Osteoid.

Introduction

Fibrogenesis imperfecta ossium (FIO) is a rare acquired abnormality of bone, first reported by Baker and Turnbull (1950) and subsequently named and described in detail by Baker (1956). Clinical features of the condition include incapacitating bone pain, bone fragility as evidenced by fractures from minimal trauma, a mottled appearance of radiographs with coarse trabeculation and raised serum alkaline phosphatase. Histological investigation shows considerable differences between FIO, normal and osteomalacic bone. The osteoid is greatly expanded, and at light microscope level appears free of fibrils and does not display the birefringence under polarized light characteristic of normal or osteomalacic bone possessing oriented collagen fibrils in lamellar arrangement (Baker 1956; Baker et al. 1966; Thomas and Moore 1968; Frame et al. 1971; Golde et al. 1971; Swan and Cooke 1973; Swan et al. 1976; Pinto et al. 1981; Lang et al. 1986). At ultrastructural level, Swan and Cooke (1973) demonstrated the presence of abnormal, distorted collagen fibers in the osteoid. The osteoid was described in more detail later (Swan et al. 1976), abnormal regions containing thin and irregularly curved collagen fibrils in a random, tangled pattern. Pinto et al. (1981) described a different arrangement, with the irregularly arranged fibers being in calcified trabeculae, and the associated osteoid being in two parts. The inner part contained some collagen fibers and numerous calcospherites, and the outer part had very sparse collagen and fewer calcospherites (see also Dopping-Hepenstal et al. 1981). Recently, Lang et al. (1986) have shown micrographs of abnormal collagen in association with varying degrees of calcification in the osteoid.

Apart from our previous report (Stamp et al. 1985), there are no reports that indicate major success of possible treatment for FIO. Certain treatments result in decrease in bone pain, but there is little consistent effect on biochemical markers and none on histology. Increased calcium retention can lead to increased bone density, however. Treatments were, in the main, based on the original work of Baker et al. (1966), using vitamin D2. This resulted in initially increased bone pain which then subsided. Resistance built up to this vitamin, however, and treatment was changed to dihydrotrachysterol, which was similarly effective. Similar results were obtained by Swan and Cooke (1973) and Swan et al. (1976), using ergocalciferol, which was changed to dihydrotrachysterol. A single report (Thomas and Moore 1968) describes a reduction in pain...
following testosterone treatment, although this was not followed up.

In this report we describe in detail the ultrastructure of FIO bone and osteoid, and demonstrate the presence of dense, abnormal collagen fibrils and sparse, two-part osteoid in different fragments of the same biopsy specimen. The case described here is the 12th to be recorded; some details of the bone ultrastructure have been published in abstract (Ali et al. 1985, 1986). Clinical details and treatment have already been described (Stamp et al. 1985). This case represents the first in which remission of symptoms following treatment can be shown to coincide with improvements in bone and osteoid ultrastructure.

Case History

The patient, a white male, presented at the age of 62 with symptoms of bone pain. Over a period of 6 years this became generalized—the patient had difficulty walking and suffered two pathological fractures. Initial investigations showed raised serum alkaline phosphatase but normal calcium and phosphate values. The patient did not respond to mineral and vitamin D supplements and was referred to the Royal National Orthopaedic Hospital. Radiographs showed a coarse, patchy appearance of bone, with coarse trabeculation. Iliac crest (IC) biopsy revealed enlarged, non-birefringent osteoid (figures taken from Stamp et al. 1985); 76% of the total trabecular surface was covered by thick osteoid (normal 14.4% ± 7.5%) and the total osteoid area was 27% of section area (normal upper limit 0.5%). Metabolic studies indicated raised acid phosphatase and a monoclonal IgG Kappa light chain band, 12 g/l rising to 20 g/l with Kappa light chains in the urine (126 μg/dl) but no Bence-Jones proteins. In addition to previous measurements, urinary hydroxyproline was also high, and the patient was in negative calcium balance. Unsuccessful experimental therapies included riboflavin, ascorbic acid, 24,25 dihydroxycholecalciferol, pyridoxine and cyclophosphamide. Mineral retention continued; however, there was no change in the clinical condition and the second IC biopsy was unaltered. After a further year, on account of rising paraprotein levels, treatment was initiated with Melphalan and prednisolone (Stamp et al. 1985). A sub-biopsy was taken from each specimen and placed in 2.5% glutaraldehyde (Agar Aids Ltd.) in 0.085M sodium cacodylate buffer, pH 7.4. This was cut into small pieces and fixation continued in glutaraldehyde for 2 h. The tissue was then rinsed in cacodylate buffer and post-fixed in 1% osmium tetroxide (Agar Aids Ltd.) in 0.085M cacodylate buffer for 1.5 hours. After a thorough wash in cacodylate buffer, tissue was dehydrated through a methanol series, immersed in propylene oxide and infiltrated with Araldite CY212 (Agar Aids Ltd.). The specimens were then embedded in fresh araldite and the blocks polymerized at 60°C for 48 h. Sections for light microscopy were cut on an LKB ultratome III ultramicrotome at 1 μm using a glass knife and were stained with methylene blue-azure II and basic fuchsin. Ultrathin sections were cut using a diamond knife; the sections spread chloroform vapor and picked up on formvar-coated 200 mesh copper grids. The sections were contrasted with saturated alcoholic uranyl acetate and Reynolds lead citrate and were examined using a Philips EM300 electron microscope at 60 kV and 80 kV accelerating voltages.

Results

The first and second biopsies, taken before successful treatment, were similar in many respects; the second biopsy did, however, reveal an additional feature of the osteoid in this condition. The third biopsy, taken after apparently successful treatment, showed considerable changes in bone and osteoid compared to the disease state.

First biopsy

At light microscope level, this specimen showed gross abnormalities of osteoid and bone (Fig. 1). The osteoid was enormously enlarged, encroaching on the marrow space and showed very limited mineralization. At ultrastructural level, the expanse of osteoid was seen to be composed of huge areas of more or less randomly oriented collagen fibrils (Figs. 2, 3). These were often curved and were irregular in diameter, ranging from 22 nm to 136 nm (Fig. 4). Occasional bundles of oriented, abnormally thin fibers were observed (Fig. 5). The noncollagenous part of the matrix consisted of moderately electron-dense granular and fibrillar material. The osteoid was not always uniformly packed with collagen fibrils; small, sparse regions were occasionally encountered (Fig. 6). Calcification was present in the osteoid in a randomly scattered calcific nodules, increasing in number towards calcified bone (Fig. 7). These may have been derived from matrix vesicles, as occasional membranous bodies were encountered in the matrix (Fig. 8). It was only at the completely mineralized front that calcification was associated with collagen fibrils (arrow in Fig. 9, see also Fig. 14). A notable feature was that sparsely collagenous regions of the osteoid matrix did not calcify in this region.

Second biopsy

Some regions of this specimen were very similar to the first biopsy, with hugely enlarged osteoid, random collagen orientation and nodular calcification, although this was present to a greater extent (Fig. 10). In another specimen
Plate 1: First Biopsy

Fig. 1. Light micrograph of 1 µm plastic section stained with methylene blue/azure A and basic fuchsine. Osteoid is densely stained, and bone palely stained. Large areas of osteoid (OS) and relatively small areas of calcified bone (b) are present. MS, marrow space; arrows, osteocytes in calcified bone. ×220. Fig. 2. Low power electron micrograph of osteoid/bone junction. Note the large area of nonlamellar osteoid (os) adjacent to calcified bone (b). ×3700. Fig. 3. Electron micrograph of osteoid. Note the random orientation and variable thickness of collagen fibrils. ×13,000. Fig. 4. Electron micrograph of uncalcified region of osteoid. Fibrils are strongly banded, with normal looking banding pattern; however they are variable in thickness, shape and orientation. ×48,000. Fig. 5. Electron micrograph of oriented bundle of collagen fibrils occasionally encountered in osteoid. Note the variation in fibril thickness. ×48,000. Fig. 6. Electron micrograph of sparse region of osteoid. Occasional patches of osteoid with sparse collagen fibrils (S) are encountered at random within the dense collagenous matrix (C). ×8200. Fig. 7. Electron micrograph of calcific nodules within osteoid. These may be derived from matrix vesicles. ×38,000. Fig. 8. Electron micrograph of matrix vesicle from uncalcified region of osteoid. ×58,500. Fig. 9. Electron micrograph of calcified collagenous matrix in deep region of osteoid (cc). Note that calcification does not occur in collagen sparse regions (s), corresponding to sparse region in uncalcified osteoid (Fig. 6) arrow, calcification associated with collagen fibers. See also Fig. 14 for a similar region at high magnification.
Plate 2: Second Biopsy

Fig. 10. Electron micrograph of calcifying region of osteoid: note large number of calcific nodules. ×34,000. In all other respects, this particular fragment resembled the first biopsy (Figs. 1–9). Fig. 11. Light micrograph of another fragment of the second biopsy (1 μm plastic section). The osteoid (os) is enlarged and shows no evidence of collagen orientation. In contrast to the previous specimens, the osteoid has two distinct regions: a densely staining deep region (d) and a less dense outer region (o). The osteoid is bounded by a layer of osteoblasts (arrows). b: bone. The boxed region is shown at EM level in Fig. 12. ×220. Fig. 12. Electron micrograph of osteoid area indicated in Fig. 11. The deep region (d) has numerous collagen fibrils (negatively stained here); the outer region appears amorphous, with few fibrillar structures being present. Osteoblasts (ob) and osteocytes (oc) have numerous cell processes extending into the osteoid matrix. ×6600. Fig. 13. Higher power electron micrograph of osteoblast. The golgi region (G) is extensive and contains many secretory vacuoles. os: osteoid side. ×14,300. Fig. 14. Calcification on deep zone of osteoid. Calcific nodules are present (arrows); in addition calcification is seen along collagen fibrils (large arrow). ×28,000.
from the same biopsy, a different but still grossly abnormal picture emerged. At light microscope level, the osteoid was similarly enlarged (Fig. 11). Huge areas surrounded vascular regions; in places, where these abutted one another there was no bone formation at all. The osteoid itself was composed of two layers of differing staining intensity.

In contrast to the other specimens the osteoblast layer and bone marrow were of normal appearance. At ultrastructural level (Fig. 12) it could be seen that the inner layer contained a network of collagen fibers, arranged in the random orientation characteristic of FIO and not ordered as in normal bone. The outer layer appeared to be amorphous and contained very little fibrillar material. Any fibrils present were generally thin and abnormal. In the deep region, cells were present which had the ultrastructural characteristics of osteocytes, but were not enclosed by calcified matrix. The osteoid was bounded by a layer of osteoblasts which were basically normal in appearance, and contained extensive rough endoplasmic reticulum and large Golgi regions (Fig. 13). The osteoblasts possessed long cell processes extending into the abnormal osteoid matrix. Calcification occurred in the deep region of the osteoid as sparsely distributed calcific nodules and in association with collagen fibrils at the completely mineralized front, as above (Fig. 14). There was no calcification in the outer region.

Third biopsy (after treatment).

Light microscopy showed the presence of lamellar bone and narrow osteoid (Fig. 15, 16). The marrow space was relatively acellular, however, and contained much dense debris. At ultrastructural level, osteoid collagen was oriented into 1-2 lamellae, as normal (Fig. 17). The fibers were of normal appearance, being straight and of uniform diameter (Fig. 18). The osteoid also showed a more normal pattern of calcification, with structures resembling matrix vesicles being present between collagen fibrils near the mineralization front; some of these appeared to contain hydroxypatite crystals (Fig. 18). The debris in the marrow space was shown to be acellular, dead bone having the abnormal collagen fiber arrangement described in the pathological osteoid of the first two biopsies (Fig. 19). Very few intact cells were present in this specimen, either in the marrow or in the osteoblast layer.

Discussion

Histological and ultrastructural features of the osteoid of specimens described here are basically in agreement with previous reports; however, certain aspects, particularly the variety of osteoid types and the appearance of the osteoblasts, have not previously been reported. The collagen morphological abnormality was as described by Swan et al. (1976), with an even wider range of fibril diameters. The nodular nature of mineralization within the osteoid has been previously reported (Dopping-Henpenstal et al. 1981; Pinto et al. 1981; Lang et al. 1986). Nodules may represent fully calcified matrix vesicles (Bernard and Pease 1969; Bonucci 1971; Bab et al. 1979; Anderson et al. 1980). It is of note that the apparently increased nodular mineralization of the second biopsy coincided with increased calcium retention following calcitriol therapy, although interpretations must be made with care owing to the small area sampled by electron microscopy. This does, however, emphasize the difference between this condition and osteomalacia: osteomalacic osteoid has a basically normal structure, and will calcify normally in the presence of adequate concentrations of calcium and phosphate; FIO osteoid is fundamentally abnormal and will not calcify as normal bone (see also Dopping-Henpenstal et al. 1981).

The mineralization defect in FIO would appear to be due to a greatly delayed calcification of collagen fibrils. Mineralization appears to be unable to spread from nodules to the rest of the matrix over the major part of the osteoid. The collagen fibrils do calcify eventually however, as shown in the deep region of the osteoid and the marrow space of the posttreatment biopsy. The necessity of collagen fibrils for embedding bone mineralization is indicated by the absence of complete calcification in collagen-poor regions of the deep osteoid. The failure of collagen fibrils to calcify promptly may be due to their structural abnormality (Frame et al. 1971; Swan et al. 1976; Lang et al. 1986), or to an abnormal relationship with their surrounding matrix. It is possible that the abnormality is caused by the large amount of amorphous material seen in the second biopsy (Fig. 2). This could interfere with fibril assembly and/or mineralization. There is evidence that this material is an osteoblast product. The presence of material in numerous Golgi vesicles within osteoblasts suggests that it may be synthesized and secreted by them; its precise nature, however, remains unknown.

The occurrence of a two-part osteoid, with a collagenous inner region and an amorphous outer region has also been described previously (Pinto et al. 1981); however, the occurrence of this and the originally described form of osteoid within the same specimen has not been reported. This shows a range of bone structure in FIO, a factor that may be responsible for the patchy or blotched appearance of radiographs (Baker 1956; Frame et al. 1971; Golde et al. 1971; Swan and Cooke 1973; Pinto 1981; Stoddart et al. 1984).

The presence of osteoblasts containing abundant endoplasmic reticulum and large Golgi regions is suggestive of active synthesis and secretion, and is a new observation. Cellular details are limited in previous reports; Pinto et al. (1981) describe inactive osteoblasts on osteoid surfaces in their material, but this is clearly not the case here. There is evidence that osteoblasts are more active in FIO than in normal bone; Hennessan et al. (1973) indicated much greater incorporation of labeled proline into collagen in FIO material compared with normal. It may be possible to explain these conflicting reports by suggesting that the osteoblasts pass through phases of activity, and that those described here were actively laying down the abnormal matrix. The previously described cells may have completed this phase. One could thus speculate that the variety of osteoid forms encountered in this disease represent different stages of development of the abnormal osteoid.

After treatment, lamellar bone with generally normal osteoid is present together with necrotic, abnormal bone in the marrow space. The abnormal osteoid evidently remains and eventually calcifies. It is replaced by bone of more normal appearance following treatment with melphalan and prednisolone. It is surprising that with the large amount of calcified matrix exposed in this material that more osteoclastic activity was not encountered. This may be explained by the hypocellular nature of the specimen due to cytotoxic drug treatment; marrow cells of all sorts were considerably depleted.

Reasons for the success of this treatment regime are not
Plate 3: Third Biopsy

Fig. 15. Light micrograph of bone fragment from posttreatment biopsy. Note discernable lamellar pattern within bone (L) and narrow osteoid seam. There is much dense debris in the marrow space (arrow). x 220. Fig. 16. Higher power light micrograph of osteoid seam and marrow space debris. x 350. Fig. 17. Low power electron micrograph of osteoid and adjacent marrow space. Note the presence of oriented bundles of collagen fibrils in osteoid and bone; some are sectioned longitudinally (L) and some transversely (T). The bone (B) is calcified; its pale appearance is due to leaching of mineral during staining. The marrow space debris (D) is highly electron dense and is calcified. x 8200. Fig. 18. High power electron micrograph of oriented collagen fibrils. Note uniformity of diameter and banding pattern. Structures resembling matrix vesicles are present between collagen fibrils; these appear to contain hydroxyapatite crystals (arrows). x 46,000. Fig. 19. Low power electron micrograph of marrow space debris. Partial demineralization during staining shows that this material contains randomly oriented collagen fibrils as in the osteoid of the pretreatment specimens. x 5100.

clear. We have already speculated that the condition is due to the effect of a marrow cell product on osteoblasts which is lost with the inhibition of marrow cells by Melphalan treatment (Stamp et al. 1985). Alternative interpretations can also be made, based on cytotoxic effects of Melphalan on osteoblasts themselves, and also on the possible effects of the glucocorticoid prednisolone given simultaneously. Cytotoxic treatment may affect abnormal osteoblast populations as well as marrow cells. An abnormal osteoblast population could be selectively removed from the tissue,
and replaced by normal cells from the narrow stromal system. A mixture of normal and abnormal stem cells in the stromal system could explain the patchy nature of the FIO bone, with normal and abnormal regions forming depending upon the nature of their original stem cell. Alternatively, treatment success could be due to glucocorticoid effects, prednisolone being given with Melphalan. While there are no specific reports dealing with glucocorticoid effects on the metabolism of human bone in vivo, there is a considerable amount of information on the effects of glucocorticoids on the skeletal system both in vivo and in vitro. In general, this involves the reduction of osteoblastic activity in the metaphysis of growing bones, in secondary spongiosa, periosteum and endosteum of growing and adult bones (reviewed by Silberberg and Silberberg 1971). More recently, in vitro results have shown specific effects of glucocorticoids on osteoblasts. Pharmacological doses of glucocorticoids, including prednisolone, have a direct inhibitory effect on collagen synthesis (Wong 1979; Wong et al. 1980). In addition, physiological concentrations of glucocorticoids increase the number of osteoblast receptors to hormones, including parathyroid hormone (Wong 1979) and vitamin D derivatives (Chen et al. 1983). It is conceivable therefore, that the glucocorticoid treatment given here along with Melphalan could have affected osteoblast metabolism. There is a single report dealing with metabolic activity of FIO bone, which describes a large increase in incorporation of radiolabeled proline into collagen when compared to normal bone (Henneman et al. 1973). One could speculate that a reduction in collagen synthesis caused by pharmacological doses of prednisolone and/or Melphalan, or mediated by prednisolone through control of hormone receptors, would have a beneficial effect on this condition. In argument against this, Henneman et al. went on to show that the usually employed treatment, using vitamin D derivatives, in fact considerably increases proline incorporation into collagen. We should like to point out, however, that vitamin D treatment does not result in significant histological change as does the therapeutic regime undertaken here; it reduced bone pain in a number of cases by an unknown mechanism, but does not affect the underlying tissue abnormality.

References


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