Histomorphology of Metaphysis of Proximal Tibia in Albino Rat

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Introduction

The metaphysis is the junctional region of bone lying between the growth plate and the diaphysis. It contains slender calcified cartilage spicules and trabecular bone and is a site of active bone turnover, having large number of osteoblasts, osteoprogenitor cells and osteoclasts amidst highly vascular tissue. The metaphysis is invaded by numerous capillary loops containing osteoblastic cells, which deposit bony matrix on calcified cartilage spicules. The metaphysis is divided into two functionally distinct regions, the primary spongiosa, the area lying adjacent to the growth plate, which is rich in blood capillaries and is the site where primary spongiosa bone forms, which is characterized by calcified cartilage spicules covered by a thin layer of newly laid bone. The other region lying adjacent to diaphysis is the secondary spongiosa, characterized by interconnecting bony bars of trabeculae. Here, the calcified cartilage spicule is ultimately resorbed by the osteoclast, and is the site of active bone remodelling. Osteoclasts, the giant multinucleated cells, are the bone resorbing cells which resorb the mineralized bone by secreting acids and lysosomal enzymes. Increased activation of osteoclasts results in disruption of normal bone remodelling resulting in increased resorption of the bone [1]. The region of metaphysis is a common site for primary bone tumours and bone infections such as osteomyelitis. The relative predilection of osteosarcoma for the metaphyseal region of long bones in children has been attributed to the rapid bone turnover due to extensive bone remodelling during growth spurts [2]. The effects of large number of drugs have also been studied on the metaphysis of long bone [3-8]. The bisphosphonates are one of the important classes of drugs which are potent inhibitors of excess osteoclastic mediated bone resorption [9].

Histomorphology of Metaphysis by Light Microscope

The histomorphology of metaphysis of proximal end of tibia was studied in young albino rats by light microscope following staining with Hematoxylin and Eosin and Masson’s trichrome. The metaphysis was identified as the area lying adjacent to the lower margin of the epiphyseal growth plate and limited on the sides by the peristemeum of the bone. The proximal margin of the metaphysis towards the growth plate was observed to be wider and more or less concavo-convex in comparison to its distal margin, which was narrow and irregular in outline. It was noted that the height of the lateral region was always more than the central region, giving a more or less concave appearance to the distal aspect of the metaphysis (Figure 1).

In Hematoxylin and Eosin stained sections, two distinct regions of the metaphysis were observed. The primary spongiosa, the area lying near the growth cartilage metaphyseal junction (GCMJ), was seen as a network of irregular, fine, thin, longitudinally oriented trabeculae. The trabeculae were connected to each other at places and separated by narrow narrow spaces containing haemopoietic tissue stained deep purple. The area near the GCMJ was characterized by a scarcity of bone marrow cells when compared to tissue found further from the GCMJ. The trabeculae were composed largely of calcified cartilage spicules covered with a thin layer of bone, the amount of bone increasing as distance from the GCMJ increased. The calcified cartilage spicules were stained darkly eosinophilic while the bony trabeculae appeared light eosinophilic (Figure 1). A large number of capillaries were seen between the calcified cartilage septae. The trabecular surfaces were lined by numerous osteoblasts and occasional osteoclasts. In Masson's trichrome stain, the bony trabeculae appeared...
dark blue while the central cores of calcified cartilage were stained light blue colour. At the junction of growth plate and metaphysis, most of the horizontal calcified walls of the lacunae of degenerating hypertrophied cartilage cells were partially eroded and longitudinally oriented calcified walls of lacunae were seen to be invaded by haemopoietic tissue, osteoprogenitor cells, osteoblasts and osteoclasts (Figure 2). The haemopoietic tissues were stained brownish red while the red blood cells took on a bright orange red colour. The secondary spongiosa, the region of metaphysis lying away from the GCMJ, was characterized by large trabeculae composed mainly of bone with occasional central cores of calcified cartilage. The newly formed bone appeared as bars of bony trabeculae of variable width and length. They were interconnected to each other at places and were arranged in a longitudinal direction (Figure 1 & 4). The surfaces of trabeculae were covered with osteoblast, osteoprogenitor cells and osteoclasts. Large marrow spaces containing marrow cells and red blood cells were seen between the trabeculae, the marrow spaces increasing further as the trabeculae of the secondary spongiosa extended towards the diaphyseal region. The occasional osteoprogenitor cells seen in secondary spongiosa were usually located adjacent to the bone surfaces. It was characterized by a poorly visualized cytoplasm. Its nucleus was its most prominent feature, being ovoid to spindle shaped, euchromatic and pale staining in appearance. Under Hematoxylin and Eosin stain, the osteoblasts were seen as a single layer of cells lining the surfaces of trabeculae and appeared cuboidal, polygonal or spindle-shaped cells having a basophilic cytoplasm with an oval, euchromatic nucleus with a single nucleolus were seen lying at one end while a prominent clear area was found at the other end of the cell. The osteoclasts were seen lying between the capillaries and trabeculae composed mainly of calcified cartilage cores. These cells were large, irregular, polymorphus and multi-nucleated with a varying number of closely packed nuclei. The nuclei were randomly placed in the foamy eosinophilic cytoplasm and were found to be round to ovoid, purple stained and usually a single nucleolus were seen in most cells while more than one nucleolus were seen in some cells. The osteoclasts were often seen lying in depressions or pits resorbed from the bone surfaces. The area surrounding the osteoclast closely applied to the trabecular surface was seen as a clear zone at places and fine tooth like extensions projecting from the cytoplasmic membrane were seen (Figure 3). The osteoclasts were found lying against the bone surfaces or sometimes lying freely within the narrow spaces. In Masson’s trichrome stain, the cytoplasm appeared reddish brown while the nuclei were stained brownish black. Oval to spindle shaped osteocytes were seen lying in clear spaces called lacunae embedded within the matrix of the trabeculae (Figure 4).

As early as in 1925, Stump [16] gave one of the earliest qualitative descriptions of the metaphysis of long bone obtained from celloidin embedded long bones of mice, rats, rabbits and sheep. The role of the calcified cartilage in providing longitudinally oriented scaffolding on which osteoblasts would form primary bone was studied. A syncitial cell population termed the ‘osteogenic mesenchyme’, was observed located adjacent to the growth cartilage metaphyseal junction (GCMJ), the point of cartilage lacunar opening and the end of the life of individual chondrocytes. The osteogenic mesenchyme was associated with a relatively high level of multiplication of young connective tissue cells and was found to invade and occupy the spaces which were earlier occupied by chondrocytes. In the osteogenic mesenchyme both osteoblasts and osteoclasts were observed.

Kimmel and Jee [17] studied the bone cell kinetics in the proximal tibial metaphysis in the rat following injection of triatitiated thymidine. Animals were sacrificed 1 to 120 hrs. Labelled osteoprogenitor cells and osteoblasts first appeared at 1 hour post-injection within 1 mm of growth cartilage metaphyseal junction (GCMJ), while labelled osteoclast nuclei first appeared at 24 hours post injection within 0.3 mm of GCMJ and was found to be depleted 5 days later, whereas that for the osteoblasts remained. The metaphysis was identified as two regions: primary spongiosa, located within 0.756 mm of the GCMJ, while labelled osteoblasts and osteoprogenitor cells. Osteoclasts were found relatively more uniformly distributed through the metaphysis than were osteoblasts and osteoprogenitor cells.
cells. The calcified cartilage disappeared at the rate of 0.0359 mm²/day; whereas bone was added at the net rate of 0.0412 mm²/day in the primary spongiosa, the only region of net addition of bone to the metaphysis. The second area composed of tissue located 1.188 mm or further from the GCMJ with an average age of 7 days or more, was a zone of much slower tissue turnover, corresponding to the secondary spongiosa. They observed a net loss of hard tissue, at a rate of 0.0090 mm²/day and the rate of loss of calcified cartilage at 0.0014 mm²/day, which was about 20 times slower than the rate of loss found in the primary spongiosa. The lower rate of turnover was correlated with the smaller number of osteoblasts and osteoprogenitor cells located in the secondary spongiosa compared to the primary spongiosa. Most osteoblasts and osteoprogenitor cells were found somewhat more scattered though the metaphysis, being relatively more numerous than osteoblasts in areas characterized by net loss of bone.

In a study on the micro vascular pattern of the metaphysis during bone growth, Aharinejad et al [18] observed that calcified cartilage formed the wall of the cylindrical compartments beneath the hypertrophied chondrocytes of the metaphyseal growth plate. These compartments ran in the bone’s longitudinal axis and contained a single capillary profile. Endothelial cells of these capillaries often showed increased cytoplasmic volume and loose texture of nuclear chromatin. Cast metaphyses by scanning electron microscope showed numerous parallel vascular loops with nodular protrusions 10–12 μm in diameter at their tips. The loops had ascending and descending limbs with a luminal diameter of 10–14 μm. Small projections 4–5 μm in diameter and delicate crests were sometimes found on the tip of the larger nodes. In a 100 × 100 μm area, there were 14–17 large nodes. By transmission electron microscope, capillary sprouts were identified at the level beneath the last row of hypertrophied chondrocytes. These capillaries had voluminous endothelial cells rich in free ribosomes and rough endoplasmic reticulum. Endothelial cell nuclei were rounded and showed loose chromatin texture. Endothelial cells were connected by intermediate junctions and there was no basal lamina. Deeper into the metaphysis, arterioles and sinusoids were observed.

As the metaphysis is a site of active bone remodelling containing mainly osteoblasts which are the bone forming cells and osteoclasts, the bone resorbing cells, it is therefore the favourite target area of bone in animal studies for observing the efficacy and potency of various drugs in experimental research and trial studies [19-24]. Animal studies have been conducted in the past to study the effects of various drugs specially bisphosphonates on the metaphysis of growing bones in rats [25, 26] and pigs [27]. As early as 1979, Miller and Jee [25] observed a marked increase in metaphyseal mineralized tissue mass as a result of slowed bone resorption in proximal tibia of rats following short-term administration of clodronate (a first generation bisphosphonate). A dramatic increase in the amount of trabecular bone was seen as compared to controls. Long term treatment of risendronate in ovariectomized induced osteopenic rats resulted in an increase in bone mass in the proximal tibial metaphysis and prevention of further bone loss in ovariectomized rats by depressing the bone resorption and turnover [26]. In pigs, long term administration of pamidronate (a second generation bisphosphonate) has shown a significant increase in trabecular bone volume and density, with a marked decrease in bone resorption [27]. Past studies have shown that bisphosphonates are highly effective in preserving bone mass in estrogen deficient rats [28] and ovariectomized adult rhesus monkey [29, 30]. Pataki et al. [31] observed that zoledronate, a third generation bisphosphonate, was highly effective in accumulating bone mass, preserving the architecture and strength than other bisphosphonates in growing rats. Bisphosphonates have now emerged as a leading therapeutic intervention for the treatment and prevention of skeletal complications of malignancy (skeletal related events) and are the treatment of choice for hypercalcaemia of malignancy, Paget’s disease of bone and postmenopausal osteoporosis [11-15, 32, 33].

References
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