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CHANGES IN TISSUE CONCENTRATION OF PROSTAGLANDINS DURING ENDOCHONDRAL BONE DIFFERENTIATION

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SUMMARY: Prostaglandins are known to be involved in bone metabolism as evidenced by the ability of PGE_2 to induce bone resorption. It was, therefore, of interest to determine if there was an association of specific prostaglandin metabolites with the various stages of developing bone by utilizing the matrix-induced endochondral bone formation system. During mesenchymal cell proliferation a peak of endogenous thromboxane B_2 was detected. In the subsequent stages of chondrogenesis and chondrolysis $PGF_2\alpha$ was in high concentration, whereas during bone formation PGE_2, 6-Keto-$PGF_1\alpha$ and thromboxane B_2 were elevated. These changes in the peak levels of the various prostaglandin metabolites may reflect differences in the cell populations and function associated with various stages of endochondral bone formation.

Direct stimulation of bone resorption in vitro by prostaglandins (1), which is associated with an elevation in cyclic AMP (2), suggests a role for prostaglandins as local mediators of physiological and pathological resorption in skeletal tissues. Moreover, the production of prostaglandins by certain cells within the bone may regulate biological functions of other cell types to bring about changes in bone metabolism. This has been suggested for osteoblasts which may directly influence the bone resorbing capability of osteoclasts by their production of PGE_2 (3). Most of the information regarding prostaglandin synthesis by osteoblasts is derived from cell

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cultures established from osteogenic sarcoma clonal lines with osteoblastic phenotype (4) and osteoblast-rich normal rat bone cells in culture (5). Another approach to studying the role of prostanoids in the developing bone is to monitor endogenous prostaglandin concentration during endochondral bone differentiation. In order to determine tissue levels of various prostanoids during discrete developmental stages, we have explored the potential of the matrix-induced endochondral bone formation system (6). This system avoids the complexity of the growth plate and enables working with tissue at discrete stages of endochondral bone differentiation. The sequential developmental changes elicited by subcutaneous implantation of demineralized collagenous bone matrix is reminiscent of long bone development and consists of mesenchymal cell proliferation, chondrogenesis, calcification and dissolution of cartilaginous matrix and vascular invasion prior to osteogenesis, followed by bone remodeling and bone marrow formation within the newly formed ossicle (6).

MATERIALS AND METHODS

Demineralized bone matrix was prepared and implanted subcutaneously into the thoracic region of Long Evans male rats (28-31 days old) as described previously (7). The day of implantation was designated as day 0. The animals were killed and plaques (implants) at various stages of development were dissected out. There were four rats in each group with two implants in each rat, yielding 8 samples for each time point. The experiments were repeated three times.

Tissue Preparation and Extraction: The tissue samples were homogenized with the aid of Polytron homogenizer in ice-cold buffer containing 0.1M NaCl, 50mM Tris-HCL and 0.1% NaN_3 at pH 7.0. Immediately thereafter ethylacetate:isopropanol:0.2N HCl (3:3:1, v/v/v) was added. After mixing thoroughly the phases were separated by centrifugation (2000 xg 10 min). The organic phase was separated, dried with nitrogen, resuspended in 10mM Tris-HCl, 140mM NaCl, pH 7.4 and assayed for various prostaglandins. The aqueous phase was collected and ice-cold 100% (w/v) of CCl_3COOH was added such that the final concentration was 10% and placed in an ice bath for 30 min. The resultant precipitate was washed twice with 10% CCl_3COOH and then hydrolyzed at 90°C for 30 min. DNA was determined in the acid hydrolysate using the diphenylamine procedure (8).

Prostaglandin Quantitation: Concentrations of PGE_2, $PGF_2\alpha$, thromboxane B_2 and 6-Keto-$PGF_1\alpha$ were determined by specific

radioimmunoassay (9). PGE_2 and $PGF_2\alpha$, were assayed utilizing rabbit anti-PGE_2 and $PGF_2\alpha$ antiserum (Miles Laboratories, Inc., Elkhart, IN), goat anti-rabbit IgG (Meloy Laboratories, Springfield, VA), ^{3}H-PGE_2 and ^{3}H-$PGF_2\alpha$ (New England Nuclear, Boston, MA) and cold PGE_2 and $PGF_2\alpha$ (Sigma, St. Louis, MO). Thromboxane B_2 and 6-Keto-$PGF_1\alpha$ were assayed by utilizing radioimmunoassay kits (American Biomedical Technologies, Nashville, TN).

RESULTS AND DISCUSSION

Measurements of the endogenous tissue concentration of various prostanoids during bone induction revealed that PGE_2, $PGF_2\alpha$, 6-Keto-$PGF_1\alpha$ and thromboxane B_2 exhibited different patterns (Fig. 1), which may suggest different roles during the discrete stages of matrix-induced endochondral bone formation. On the first day postimplantation, when the implants consisted mainly of fibrin and polymorphonuclear leukocytes, the levels of all the metabolites were low. On day 3 during mesenchymal cell

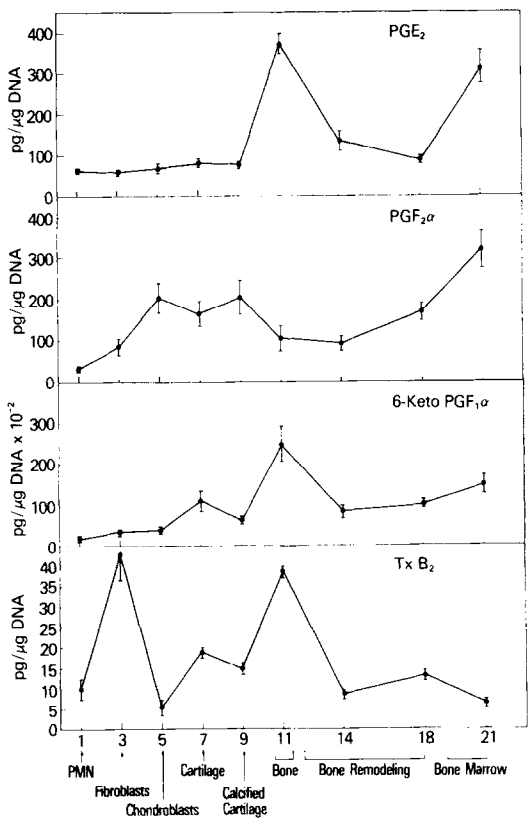

Fig. 1. Tissue concentration of various prostaglandins during endochondral bone development.

proliferation, but prior to chondrogenesis, the thromboxane B_2 level peaked suggesting that this prostaglandin may play a role during the mitogenesis occurring in response to the implanted collagenous matrix (10). Thromboxane B_2 has also been implicated in the regulation of mitogenesis of human lymphocytes (11). The elevation of $PGF_2\alpha$ on days 5 to 9 post implantation during chondrogenesis, calcification and dissolution of cartilaginous matrix and the lower levels during bone formation may imply a special role for this prostaglandin. This pattern suggests that chondrogenesis and chondrolysis in particular, may be $PGF_2\alpha$ and not PGE_2 dependent, and in fact only very small quantities of $PGF_2\alpha$ were found to be synthesized by osteosarcoma cells in culture (12). On day 11 when the implants consisted mainly of osteoblasts, all measured prostanoids attained peak levels except $PGF_2\alpha$. The appearance of osteoblasts was marked by a 4-fold increase in PGE_2 levels. PGE_2 accumulation at this stage in the tissue supports the Rodan and Martin hypothesis (3) implicating a role for osteoblasts in bone resorption. The lower levels during the bone remodeling phase up to day 18 may be explained by rapid utilization or clearance of this local mediator. 6-Keto-$PGF_2\alpha$, the stable breakdown product of prostacyclin, has been shown to be a major product of arachidonic acid metabolism by clonal osteosarcoma cells (12). Although both PGE_2 and prostacyclin are known to stimulate adenylate cyclase activity in osteoblasts (5) and activate cyclic AMP-dependent protein kinase (12), the former is more potent. The significantly lower tissue levels of 6-Keto-$PGF_2\alpha$ relative to PGE_2 may suggest a different role if not a different cellular origin, such as endothelial cells (13). It is well known that vascular invasion is a prerequisite for bone formation (14-15). Thromboxane B_2, which appears not to be synthesized by osteoblast-like cells in culture (12), was

elevated again on day 11. So far there is no evidence for its origin or function in skeletal tissue, although there is evidence that endothelial cells in culture produce both thromboxane B_2 as well as prostacyclin (16). During bone marrow development within the newly formed ossicle (6,7), active proliferation and differentiation of hematopoietic cells occur and may account for the second elevation in PGE_2 levels as well as for the accumulation of $PGF_2\alpha$ and 6-Keto-$PGF_1\alpha$. The data presented here indicate that there are significant changes in various prostaglandins during discrete stages of endochondral bone development. Further experiments on the endogenous metabolism and the precise local regulation of prostaglandins in situ may shed more light on their role in normal bone formation and remodeling.

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