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Studies of Human Osteoblast-like Cells; - Effects of Growth Hormone and Steroids Diana Swolin-Eide, M.D., Ph.D.

Address:
Research Center for Endocrinology and Metabolism,
Department of Internal Medicine
Sahlgrenska University Hospital,
SE-413 45 Göteborg, Sweden
or
Pediatric Growth Research Center,
Queen Silvia Children's Hospital,
Sahlgrenska University Hospital,
SE-416 85 Göteborg, Sweden

Email: diana.swolin@medic.gu.se

Introduction Bone tissue is a metabolically active organ which is continuously remodelled. The regulation of bone resorption, bone formation and interactions between different hormones and cytokines in human osteoblasts is not completely understood. Growth hormone (GH) is important in determining final body height and for normal bone physiology. High levels of glucocorticoids result in osteoporosis, while oestrogen has a protective effect on bone mass. Interleukin-6 (IL-6) and interleukin-1 (IL-1) are two cytokines which are believed to be of importance for the local regulation of bone remodeling. This report is a summary of my thesis in which I investigated the effects of GH, oestrogen and cortisol and their interactions with each other and with interleukins in vitro in primary isolated human osteoblast-like cells[1].

Bone Tissue and Bone Remodeling The skeleton has a protective role for vital organs; it acts as a supporting frame for muscles and connective tissue and is the important location for haematopoiesis. It also serves as a reservoir of calcium and other ions, like phosphate and magnesium. There are two different kinds of bone: cortical and cancellous (trabecular) bone. Cortical bone is compact and found mostly in long bones as a shell, whereas cancellous bone consists of a network of bone trabeculae and is found mostly in the vertebrae and pelvis. Cancellous bone is more metabolic active with a larger surface area and is, therefore, more susceptible to bone resorption[2].

Bone forming cells, osteoblasts (OBs), are derived from mesenchymal stroma precursor cells in the bone marrow. The cells actively secrete the extracellular matrix on one side of the cell3. To qualify as an OB, cells are required to display some part of the characteristics that are typical for OBs. These phenotypical characteristics include: the synthesis of collagen type I, the expression of alkaline phosphatase (ALP) activity, the expression of osteocalcin, intracellular cAMP stimulation by parathyroid hormone and the ability to mineralize the extracellular matrix, osteonectin, osteopontin and vitamin D receptors[3, 4]. Several different isolation and culture methods have been used to study OBs in vitro, mainly confined to chicken, rabbit, and rodent tissue or transformed cells. In the 1980s Crisp et al.[5] and MacDonald et al.[6] reported new methods for isolating primary OBs from human cancellous bone, which was an important step towards understanding human bone physiology better. The cells obtained by using these culture methods are not transformed and display osteoblastic phenotype. Today, two different methods are mainly accepted for the isolation of human osteoblast-like cells (OBs); with enzymatic digestion[7] or without enzymatic digestion treatment[5, 6] of bone chips.

The cell responsible for the resorption of bone matrix is the osteoclast (OC). This is a large, multinucleated cell which is believed to be derived from haematopoietic stem cells in the bone marrow. Osteoclasts are asymmetric cells, having a ruffled border region which is an area where active resorption takes place[8]. The skeleton was for many years regarded as an inactive tissue but today it is regarded as a metabolically active and dynamic tissue. The continuous removal of bone (bone resorption) by OCs and the following synthesis of new bone matrix and its subsequent mineralization (bone formation) by OBs is a process called bone remodeling, see[19,10] Fig. . The process starts when OBs or OB-derived cells (bone lining cells) digest the uncalcified osteoid and, thereby, expose the mineralized bone surface. Precursors of OCs then start to differentiate into mature OCs, which attach to the bone surface and start to resorb bone. This bone resorption is followed by a reversal phase during which the OCs are replaced by other cells. Furthermore, pre-OBs proliferate and start to form new unmineralized bone matrix, referred to as osteoid, which is subsequently mineralized. This phase is then followed by a quiescent phase before a new bone remodeling cycle starts again[2, 9].

The process by which bone resorption is followed by bone formation is called coupling and is ensures that the bone removed is replaced by new bone. Thus, coupling secures the balance between resorption and formation. Osteoporosis is a systemic metabolic bone disease where there

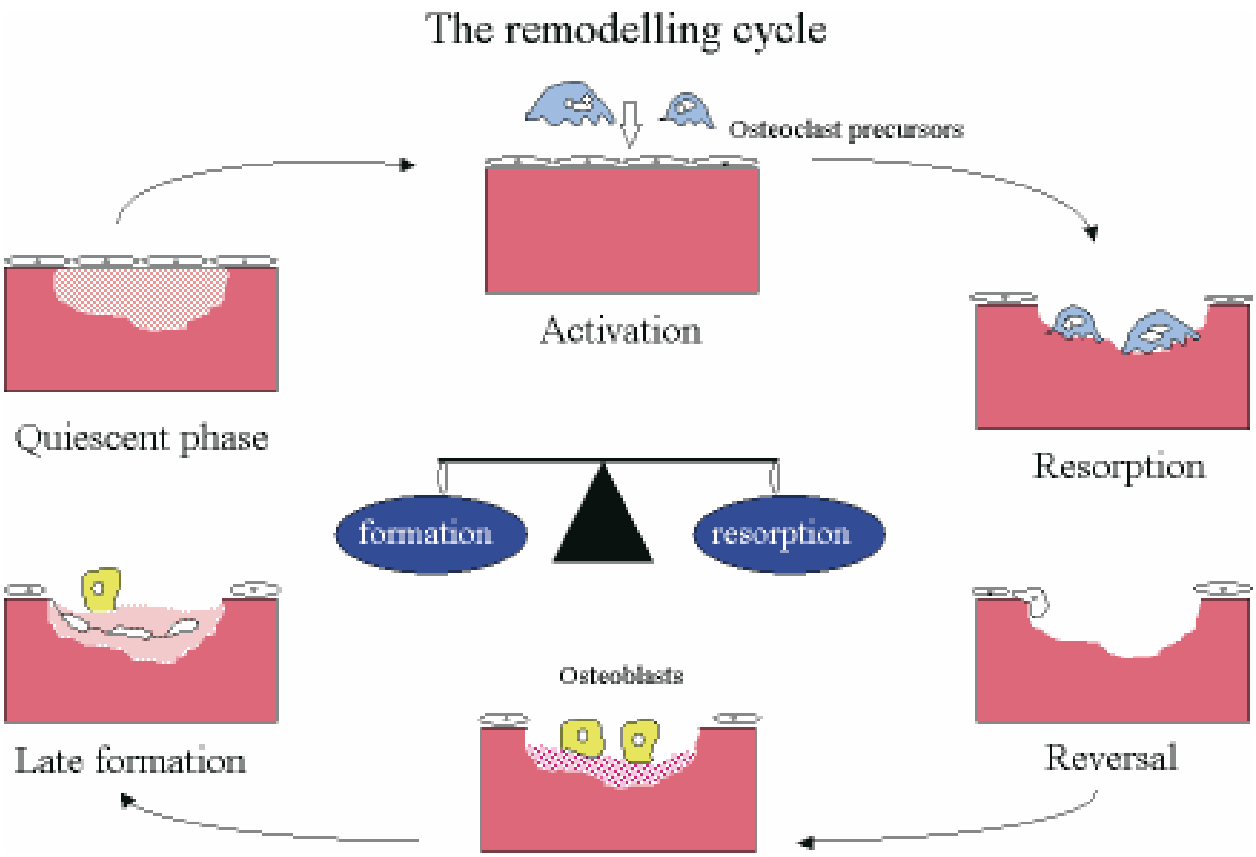

Figure 1 - The Bone Remodelling Cycle

is an imbalance in the remodelling cycle. The definition of osteoporosis, according to the World Health Organization, is when the bone mineral density (BMD) is >= 2.5 standard deviations below peak bone mass (the maximal bone mass) [11]. The clinical consequences of osteoporosis are fractures, mainly affecting the spine, the hip and the forearm.

Growth Hormone Acts Directly on Human Osteoblast-like Cells

It is well known that GH is important in determining longitudinal bone growth and for normal bone remodeling[12, 13, 14]. Our group demonstrated, for the first time, that normal primary isolated human OBs express functional GH receptors[15]. This finding is consistent with earlier findings by Barnard et al.[16] and Slootweg et al.[17] who demonstrated functional GH receptors on rat osteosarcoma cells and on mouse OBs. The number of GH-binding sites was lower in human OBs (approximately 2000) than in rat osteosarcoma cells (9000). The lower number of GH-binding sites in human OBs compared with rat osteosarcoma cells may be due to the fact that human OBs are a heterogeneous cell population of primary cells and/or might reflect a species difference. Furthermore, the high expression of GH-binding sites in rat osteosarcoma cells may be a consequence of the transformed phenotype. As OBs express functional GH receptors, GH can act directly on bone. GH has been shown to increase GH receptor mRNA levels and GH-binding in rat epiphyseal chondrocytes[18] and in mouse OB cells[17].

GH is known to be anabolic for OBs and to stimulate the proliferation of cultured OBs. Some studies, but not all, demonstrate that GH regulates the differentiation of cultured OBs[19, 20, 21, 22]. GH was found to increase the proliferation but not differentiation (ALP activity) of human OBs[15]. The lack of effect on ALP activity may be due to the culture conditions[23]. Another explanation can be that human OBs are a heterogeneous cell population, consisting of cells at different stages of differentiation. There are, according to Stein et al.[24], well established variations in the competency of OBs to respond to different hormones throughout differentiation.

There are several signs that Insulin-like growth factor 1 (IGF-1) is important in bone remodeling and that IGF-1 is a factor which is embedded in the bone matrix and can act as a coupling factor between bone formation and bone resorption[25]. We demonstrated[26] that human OBs express IGF-1 mRNA and this is similar to results obtained simultaneously by Okazaki et al.[27]. This finding shows that IGF-1 is locally produced by OBs.

The somatomedin hypothesis states that GH stimulates the production of IGF-1 in the liver, and that the liver-produced IGF-1 then stimulates longitudinal bone growth in an endocrine manner[28]. Another theory for the effect of GH on longitudinal bone growth is the “dual effector theory” of Green et al.[29] which was adopted for longitudinal bone growth by Isaksson et al.[30]. This theory suggests that GH stimulates the differentiation of mesenchymal precursor cells and then that locally produced growth

factors like IGF-1 promote the clonal expansion of more differentiated cells. There are some findings that suggest that the dual effector theory of GH action may at least partly be valid for osteoblastic bone formation. It has been demonstrated in rodent OBs that the mitogenic effect of GH is blocked by an anti-serum to IGF-1 and that GH induces IGF-1 expression in OBs[20, 31], whether or not local IGF-1 is regulated by GH is still unclear in human OBs[14]. GH and IGF-1 may also have synergistic effects regarding growth-promoting activity in rats[32].

Malpe et al.[33] published results indicating that there are skeletal site-dependent differences in the production of IGF. Skeletal site differences suggest that the regulation of bone metabolism may vary between different skeletal sites. Furthermore, there are a number of reports which show that the action of GH on bone formation is site dependent. GH treatment results in a subperiosteal cortical bone formation, while no or minor effect is found on cancellous bone[14,34].

Growth Hormone and Interleukin-6

The multifunctional cytokine IL-6 is involved in bone remodeling. The osteosarcoma cell line Saos-2 expressed very low levels of IL-6[35, 36], whereas the expression in the osteosarcoma cell line MG 63 was similar to that found in human OBs. This finding indicates that various cell lines differ in their expression of cytokines and that one cannot always extrapolate results from transformed cell lines to normal OBs. The effect of GH on the production of IL-6 in human OBs has been investigated. GH increased IL-6 expression in a dose- and time-dependent manner in human OBs[35]. Similar results have previously been demonstrated in chondrocytes[36]. As the effect of GH on IL-6 expression is major, one could assume that there is a physiological function for this regulation.

One may speculate that GH interacts directly with the OBs to stimulate them to produce IL-6 and, via an increased IL-6 production, induce OC differentiation which in turn results in increased bone resorption. An alternative effect for IL-6 induced by GH, is suggested by studies demonstrating that IL-6 promotes the differentiation of OBs[37] and that IL-6, in the presence of its soluble receptor, induces the differentiation of uncommitted embryonic fibroblasts towards cells of the osteoblastic lineage[38]. Thus, these studies indicate that IL-6 induced by GH could be of importance to bone formation.

Effects of Oestrogen on Growth Hormone Action and Growth Hormone Receptor Expression in Human Osteoblast-like Cells

Oestrogen is important to maintaining a normal balance in bone remodeling. A severe decrease of serum oestrogen levels after the cessation of ovarian function leads to postmenopausal osteoporosis. As GH is an important factor in the regulation of bone mass, it is of interest to study a possible interaction between oestrogen and GH at the cellular level in human OBs. Slootweg et al.[39] demonstrated the interaction of oestrogen and GH with regard to their proliferative effects on OBs. Using certain culture conditions, neither GH nor oestrogen stimulated cell proliferation. Interestingly, when both hormones were administered together, an increase in proliferation was observed. The lack of proliferative response to oestrogen alone in OBs coheres with results from Rickard et al.[40] and Keeting et al.[41]. Other groups have reported that oestrogen stimulates or inhibits the proliferation of OBs[42].

Oestrogen was found to stimulate both GH-receptor mRNA levels, as well as GH binding. This increase in GH-receptor expression was found in both human OBs and in rat osteosarcoma cells[39]. However, the dose-dependent effects of oestrogen on GH receptor expression are somewhat different between the human OB cells and the UMR cells. This difference could be a result of species difference and/or that the two cell types express different amounts or subtypes of the oestrogen receptor. The finding that oestrogen regulates GH receptor expression is supported by results from Gabrielsson et al.[43], demonstrating that oestradiol upregulates GH receptor mRNA levels in rat liver. From the studies by Sandstedt et al.[44] it is concluded that elevated levels of GH increase the amount of vertebral as well as tibial bone in young female mice and that intact ovaries are a prerequisite for the stimulatory effect of elevated GH levels. Furthermore, a clinical study with acromegalic women demonstrated that the anabolic effect of GH on bone is more evident in the presence of oestrogens[45]. Together, these findings suggest that oestrogen modulates the GH response in vivo as well as in vitro. Some of the synergistic effects between GH and oestrogen may be explained by the fact that oestrogen increases the number of GH receptors. One may speculate that a combined treatment with oestrogen and GH could be useful in the treatment of postmenopausal osteoporosis.

Effects of Glucocorticoids on Human Osteoblast-like Cells

Cortisol is another hormone which is involved in bone remodeling and in the pathogenesis of osteoporosis[46]. Cortisol exerts complex effects on bone tissue and on bone cells. It is well known that high levels of cortisol decrease bone formation. Some studies indicate that high levels of cortisol also result in increased bone resorption. It is important to distinguish between high (pharmacological) doses and low (physiological) doses of cortisol treatment. Low doses of cortisol are mostly anabolic while high levels of cortisol are catabolic for bone tissue. High levels of glucocorticoids result in decreased collagen expression and in an increase in collagenase expression, which leads to the degradation of type I collagen[47]. Cheng et al.[48] have shown that glucocorticoids stimulate the differentiation of human bone marrow stromal cells into OB cells. A positive effect of physiological doses of glucocorticoids is that they promote a more differentiated OB phenotype[49]. We have shown that a low dose of hydrocortisone increases cell proliferation and ALP activity in human OBs[15]. This increase in cell proliferation is similar to what Jonsson et al.[50] have shown for short-term treatment with low dose of glucocorticoids. They also found that glucocorticoids stimulation resulted in a biphasic effect on proliferation, where a more prolonged glucocorticoids period with high doses of glucocorticoids were found to inhibit proliferation, reflecting the complex

mechanism of action for glucocorticoids on bone cells. However, most studies demonstrate that prolonged treatment with high levels of glucocorticoids is catabolic for OBs.

The effect of cortisol on GH receptor expression has been studied by us in human OBs. Unexpectedly, it was found that high levels of cortisol increased GH receptor expression. Both GH-receptor mRNA levels and GH-binding were increased by high doses of cortisol51. One might have assumed that cortisol would have decreased GH receptor expression and, thereby, exerted a negative effect on bone formation. However, these findings are similar to in vivo results in which glucocorticoids increased GH-receptor mRNA levels in the liver and growth plate of rabbits[52] and in rat osteosarcoma cells where glucocorticoids increased GH binding[53]. Interestingly, Salles et al.[53] found that the GH receptor expression was enhanced by glucocorticoids but the stimulatory effect of GH on the proliferation of rat osteosarcoma cells was partially blocked by a high dose of dexamethasone. These findings suggest that glucocorticoids block the GH effect at a post-receptor level. Future studies will determine whether or not high levels of glucocorticoids block the GH-response at a post-receptor level in human OBs.

Another mechanism by which cortisol regulates bone metabolism may be via a regulation of IGF expression. IGFs exert anabolic effects on OBs[47]. An anabolic effect of IGF-1 is supported by the finding that IGF-1 increased human OBs cell-proliferation and ALP activity[15]. Interestingly, cortisol inhibits the expression of IGF-1 mRNA in human OBs[26] and similar results have previously been obtained in fetal rat OBs[54]. The finding that high doses of cortisol decrease the IGF-1 expression in human OBs is one possible, and maybe important, mechanism by which cortisol exerts its inhibitory actions on bone formation. Further evidence that decreased IGF-1 expression maybe important to a glucocorticoid-induced decrease in bone formation, is a study by Jonsson et al.[55]. The study demonstrates that a high dose of hydrocortisone inhibits the release of carboxyterminal propeptide of type I collagen into the culture medium of human OBs. The addition of IGF-1 normalized the release of carboxyterminal propeptide of type I collagen from the hydrocortisone incubated human OBs. This finding indicates that IGF-1 has the capacity to reverse the negative effects of cortisol on bone formation. In conclusion, in vitro data indicate that IGF-1 may be a potential anabolic substance for the treatment of glucocorticoids-induced osteoporosis.

To further investigate the effects of glucocorticoids on human OBs, the interaction between cortisol and IL-1 and IL-6, two cytokines that are involved in bone remodeling, has been studied. We demonstrated that the expression of these two interleukins is decreased by high doses of cortisol[56]. Similar results have earlier been presented in studies using mouse OBs[57]. Furthermore, dexamethasone inhibited the release of IL-6 in human bone marrow stromal osteoprogenitor cells[58]. The observations that cortisol decreases IL-6 and IL-1b expression in OBs are somewhat surprising, as IL-6 and IL-1, as well as cortisol, have been regarded as factors which promote bone resorption. Thus, cortisol-induced bone resorption cannot be explained by a cortisol-induced decrease of IL-6 and IL-1 production in OBs. However, some studies indicate that IL-6 and IL-1 may have a function as positive modulators of bone formation. The finding that cortisol reduces the production of IL-6 and IL-1 in human OBs may also be a part of a generally applicable biological feed-back mechanism for regulating the production of cytokines and not a major determinant for cortisol-induced osteoporosis.

In conclusion, the in vitro model by using primary isolated human osteoblast-like cells, contributes to increasing knowledge of basal mechanisms in human bone physiology. The human osteoblast is a cell which is highly affected by different hormones, cytokines and growth factors. The regulation of all these substances have to be further studied as well as all the secrets of the osteoblasts. The new information may hopefully result in development of new treatment strategies for patients with osteoporosis, growth disorders and metabolic bone diseases.

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