COMMENTARY

Glucocorticoid activity, inactivity and the osteoblast

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Introduction

Although excess circulating glucocorticoids have detrimental effects on bone health, the processes underlying these effects are not well understood. We review what is currently known about the effects of glucocorticoids on osteoblast proliferation and differentiation, with particular emphasis on osteoblast expression of glucocorticoid metabolic enzymes as a mechanism for regulating corticosteroid activity in bone.

The adverse effects on bone of increased circulating concentrations of endogenous glucocorticoids have been recognised since Harvey Cushing's original description of Cushing's disease (Cushing 1912). The increasing use of synthetic glucocorticoids for inflammatory diseases has increased the pressure to understand, and ultimately modify, the processes underlying their deleterious effects on bone. The clinical impact of the problem is reflected both by a doubling of the overall fracture risk in patients taking prednisolone (7·5 mg for 6 months) and the development of low bone mineral density in 50% of patients taking higher doses of glucocorticoid (Eastell 1995). Several mechanisms have been proposed for these effects, including reduction in endogenous sex steroid production, decreased muscle mass, and altered gastrointestinal and renal calcium handling causing secondary hyperparathyroidism (Canalis 1996). However, it appears increasingly likely that the most important effect of glucocorticoids is to alter the proliferation and differentiation of cells of the osteoblast lineage. Bone formation *in vivo* is clearly a complex dynamic process, involving the actions of multiple morphogens, growth factors and hormones. Glucocorticoids could affect any part of the differentiation pathway from initial skeletal patterning and mesoderm induction, commitment of primitive multipotent mesodermal progenitor cells to the osteoblast lineage, or the proliferation and differentiation of preosteoblasts through to mature osteoblasts, to bone-lining cells and osteocytes (Fig. 1). All these stages are likely to occur in normal bone turnover and during fracture healing. For each of these stages, the effects of glucocorticoids, their mechanisms of action and, equally important, the reasons for their lack of action, upon other osteoblasts within the bone microenvironment require explanation. These are outlined in the following review, with particular emphasis on osteoblast expression of glucocorticoid metabolic enzymes as a mechanism for regulating steroid activity in bone.

Glucocorticoids and the commitment of multipotent mesodermal cells to the osteoblast lineage

Although the effects of glucocorticoids on the formation and proliferation of the earliest osteoblast progenitors potentially have the greatest consequences for the ultimate integrity of bone tissue, remarkably little is known about these processes. Glucocorticoids exert a permissive effect *in vitro* on cultures of marrow stromal and osteoblast cells. They also affect bone morphogenetic protein expression and can interact with bone-specific nuclear transcription factors.

Glucocorticoids increase the number and size of bone nodules formed in primary bone cell cultures (Cheng *et al.* 1994). This appears to be due to stimulation of a distinct subset of glucocorticoid-sensitive progenitors. It is not yet clear whether these cells are relatively more primitive or more mature, but osteogenic differentiation in marrow stromal, rather than osteoblast, cultures is even more dependent on the effects of glucocorticoids (Bellows *et al.* 1990), suggesting that it is the earlier progenitors that are stimulated. The properties of this subset of cells that explain their sensitivity to glucocorticoids remain unknown, as do those that explain the insensitivity of the other cells within the culture.

A potential mechanism for these effects is glucocorticoid modulation of expression and action of bone morphogenetic proteins (BMPs). BMPs are secreted polypeptides, many of which can induce cartilage and bone formation when injected subcutaneously or intramuscularly. Most are members of the transforming growth factor (TGF) β superfamily, bind to specific cell-surface BMP receptors and alter gene expression by phosphorylating Smad proteins (specifically Smad1 and Smad5) (Sakou 1998). BMPs-2 and -4 cause a dose-dependent differentiation

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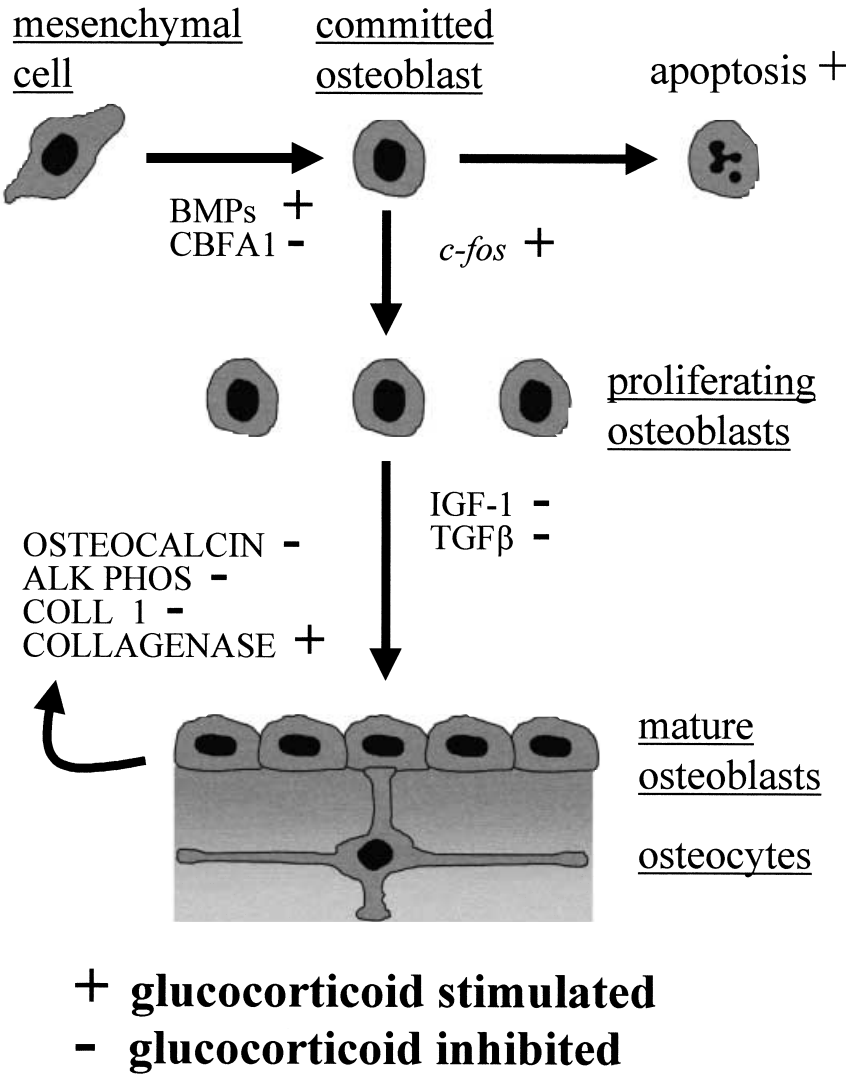

Figure 1 Regulation of proliferation, differentiation and function of cells of the osteoblast lineage by stage-specific factors. Glucocorticoids appear to have actions (stimulatory +, inhibitory −) on all these regulatory factors. ALK PHOS, alkaline phosphatase; COLL, collagen.

of mouse mesodermal progenitor cell lines to cartilage and bone, and BMP-2 promotes differentiation of cells representing early differentiated osteoblasts. Recombinant human BMP-2 has been successfully used as an adjuvant to bone grafting procedures in experimental animals, whereas BMP-6 potently increases bone nodule formation *in vitro* and ectopic bone formation *in vivo* when produced by tumours. Glucocorticoids and BMP-2 have been shown to exert a synergistic induction of osteoblast differentiation in both marrow cell cultures (Rickard *et al.* 1994) and a fetal rat calvarial cell culture model (Boden *et al.* 1996). Glucocorticoids also increase the expression of both BMP-6 protein and mRNA several-fold in the calvarial model. Similarly, antisense BMP-6 treatment can partly block glucocorticoid-induced differentiation (Boden *et al.* 1997). This suggests that glucocorticoid modulation of BMP expression and action is likely to be an important factor in regulating osteoblastic differentiation.

In addition to BMPs, bone formation is critically dependent upon the expression of the nuclear transcription factor CBFA1 (core binding factor-1) (Komori *et al.* 1997). Homozygous disruption of the CBFA1 locus in mice leads to normal cartilage formation, but an absence of bone tissue. Expression of CBFA1 is upregulated by BMP-2 and the normal expression of bone specific markers in

BMP-2-treated marrow cultures is blocked in CBFA1-/- mice. Glucocorticoids (both cortisol and dexamethasone) have been shown to suppress the effects of CBFA1 on TGFβ receptor 1 promoter activity (Chang *et al.* 1998) possibly by decreasing CBFA1 mRNA stability. Thus glucocorticoids can suppress an effect that would be expected to be anabolic to bone. The combination of effects on BMPs and CBFA1 implies that glucocorticoids can have opposing effects on the induction of osteoblast commitment, inducing and potentiating the effects of BMPs on the one hand whilst suppressing the effects of CBFA1 on the other. The resultant effect *in vivo* remains to be determined, as does the importance of continued CBFA1 expression in committed osteoblasts and whether the suppressive effect of glucocorticoids continues.

Glucocorticoids and proliferation, differentiation and function of committed osteoblasts

Glucocorticoids affect both proliferation and differentiation of osteoblasts, and their effects are often biphasic within the same system. Most studies have focused on *in vitro* systems. In cultured fetal rat calvariae, physiological concentrations of cortisol have a stimulatory effect on collagen synthesis at 24 h, whereas supraphysiological concentrations have an inhibitory effect at 48–96 h. The stimulatory effects appear to be due to increased osteoblastic differentiation, whereas the inhibitory effects result from a reduction in proliferation (Gronowicz *et al.* 1994). Direct evidence for changes in proliferation and differentiation of osteoblasts *in vivo* is sparse. One study showed that bone marrow cultures from mice treated with pharmacological concentrations of glucocorticoids showed decreased formation of fibroblast colony-forming units, possibly indicating a depletion of osteogenic precursors (Simmons *et al.* 1990). There is clearly a need for better *in vivo* model systems.

Glucocorticoids affect several osteoblast-specific and osteoblast-related gene products, including proto-oncogenes, collagens and collagenases, bone matrix components and potential growth factors. The earliest glucocorticoid mediated effect seen *in vitro* is a rapid transient increase in the proto-oncogene, c-fos. Expression of c-fos is greatest during osteoblast development, and overexpression of c-fos in transgenic mice leads to the development of multiple osteoblastic and chondrogenic tumours (Grigoriadis *et al.* 1993), suggesting an important role in the regulation of osteoblast differentiation. This effect is not seen with overexpression of other proto-oncogenes, such as c-jun. The c-fos is an important component of activator protein-1 (AP-1) trans-regulatory complex, which can influence expression of many osteoblast related genes. In contrast to the stimulatory effect on c-fos, glucocorticoids have an inhibitory effect on AP-1-mediated activity in many systems, an effect believed to occur through direct protein–protein interactions between AP-1 and the glucocorticoid receptor. As with many of these gene products, the relevance of glucocorticoid-induced c-fos expression or AP-1 inhibition in bone *in vivo* is unclear.

Type 1 collagen is the major constituent of bone matrix, and glucocorticoids have important effects on type 1 collagen expression. There is a decrease in both protein and mRNA levels in rat and human osteoblasts and in rat calvarial cultures treated with dexamethasone (Delany *et al.* 1994). In addition, glucocorticoids may alter expression of enzymes that degrade collagen. Collagenase is expressed in normal human osteoblasts *in vivo* (Bord *et al.* 1996) and expression in cultured rat calvarial cells *in vitro* is stimulated by glucocorticoids (Delany *et al.* 1995). Osteoblast-mediated collagenase activity has been implicated in the removal of unmineralised osteoid that is lining bone surfaces (Chambers & Fuller 1985). This action may initiate the bone remodelling cycle, and excess stimulation of this activity by glucocorticoid could have a deleterious effect on bone.

Glucocorticoids also affect the non-collagenous components of bone matrix. Osteocalcin is the most abundant of these, although its function remains unclear. In most osteoblastic systems studied, glucocorticoids inhibit expression of osteocalcin mRNA and protein, an effect that also occurs *in vivo*. This effect is believed to occur by binding of glucocorticoid receptor to a response element in a region overlapping a TATA box in the osteocalcin promoter (Stromstedt *et al.* 1991). A further important marker of osteoblastic differentiation and growth is alkaline phosphatase. Alkaline phosphatase expression is usually upregulated by glucocorticoids *in vitro*, as a result of a direct transcriptional effect. *In vivo*, however, glucocorticoids cause suppression of alkaline phosphatase in mature osteoblasts. As with many glucocorticoid responses, the reason for stimulation in one context and suppression in another remains unclear.

Glucocorticoids also affect osteoblasts through changes in the concentration of, or sensitivity to, other growth factors or hormones. This has been investigated most extensively for insulin-like growth factor (IGF)-1 and TGFβ. IGF-1 is mitogenic for osteoblasts and anabolic for bone. Glucocorticoids decrease expression of IGF-1 mRNA expression in rat tibia, fetal rat calvarial cultures and primary osteoblastic cells (Chen *et al.* 1991). They have complicated and diverse actions on IGF binding proteins (IGFBPs), but appear to reduce the concentration of several IGFBPs (Canalis 1996). Glucocorticoids may also interfere with IGF-1 signalling at a post-receptor level. TGFβ is another anabolic growth factor for bone. Glucocorticoids decrease the stimulatory effect of TGFβ on fetal rat osteoblastic cells and increase the binding of TGFβ to betaglycan (Centrella *et al.* 1991), the low-affinity TGFβ receptor that may cause a sequestration of TGFβ and therefore a decrease in activity. In contrast,

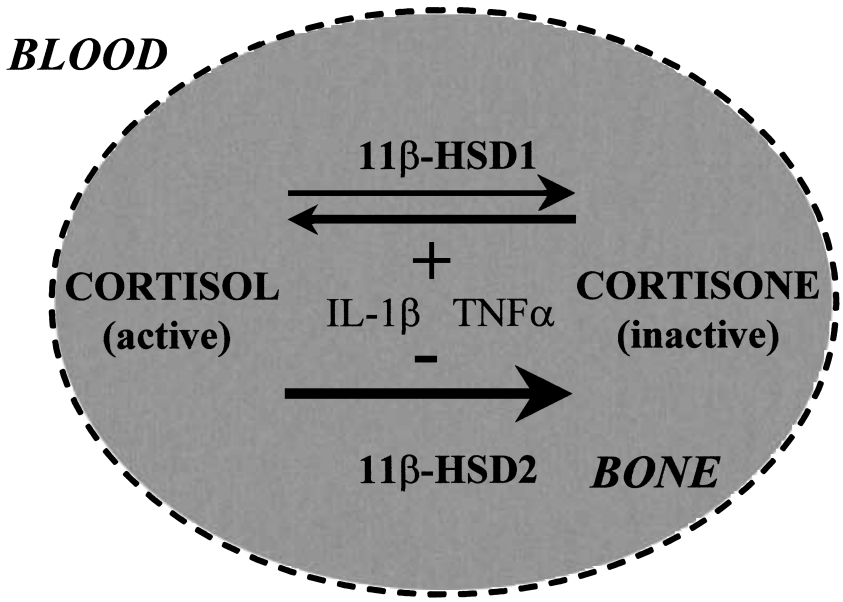

Figure 2 Schematic illustration of the role of 11β-HSD enzymes in the osteoblast. Glucocorticoid hormone action is likely to be dependent on the balance between intracellular glucocorticoid production and inactivation. This balance is likely to be regulated by differentiation stage and factors present within the bone microenvironment. IL, interleukin.

glucocorticoids increase the formation of active TGFβ from latent TGFβ (Oursler *et al.* 1993). The functional consequences of these changes in the IGF-1 and TGFβ axes *in vivo* are unknown.

In addition to these effects on proliferation, differentiation and function of osteoblasts, the role of glucocorticoids in the induction or inhibition of apoptosis is likely to be important. An increase in the rate of apoptosis of both osteoblasts and osteocytes in adult mice treated with prednisolone has been demonstrated (Weinstein *et al.* 1998) but, in contrast, glucocorticoids were protective against apoptosis induced in primary human osteoblasts by activated peripheral blood mononuclear cells (Nakashima *et al.* 1998). These opposing results suggest that the sensitivity of the osteoblast to the action of glucocorticoids is critically dependent on its state of differentiation.

Mechanisms for alteration of osteoblast sensitivity to glucocorticoids

Several mechanisms have been proposed whereby the cellular sensitivity to glucocorticoids can be altered. These include changes in affinity or concentration of glucocorticoid receptor, alteration in the ability of the receptor to dissociate from heat shock proteins, undergo phosphorylation or translocate to the cell nucleus, and modulation of the interaction of the glucocorticoid receptor with glucocorticoid response elements, other transcription factors or the basal transcription machinery (Bamberger *et al.* 1996). In the osteoblast, these mechanisms have not been studied in any detail. At a pre-receptor level, the functional importance of intracellular glucocorticoid metabolism in several tissues such as kidney (Stewart *et al.* 1996), liver (Kotelevtsev *et al.* 1997) and omental fat (Bujalska *et al.* 1997) has recently been demonstrated. 11β-Hydroxysteroid dehydrogenase (11β-HSD), by catalysing the interconversion of hormonally active cortisol to inactive cortisone, is established in these tissues as an important modulator of corticosteroid hormone action. Two isozymes of 11β-HSD, 11β-HSD1 and 11β-HSD2 have, respectively, been shown to regulate glucocorticoid and mineralocorticoid hormone action (White *et al.* 1997). 11β-HSD1 is a low-affinity, bi-directional, NADPH-dependent enzyme that has a greater affinity for cortisone than for cortisol. In contrast, 11β-HSD2 is a high-affinity, NAD-dependent dehydrogenase, inactivating cortisol to cortisone. In addition to endogenous glucocorticoids, the synthetic glucocorticoids, prednisolone and dexamethasone, are substrates for 11β-HSD, though with reduced affinity compared with cortisol and cortisone. We have recently demonstrated the presence of 11β-HSDs in human osteoblasts: 11β-HSD1 in osteoblast primary cultures (Bland *et al.* 1999), cell lines and normal adult bone (Cooper *et al.* 1998); 11β-HSD2 in osteosarcoma cell lines (Bland *et al.* 1999) and normal fetal bone (Condon *et al.* 1998). 11β-HSD expression and activity are regulated by factors likely to be present in the bone microenvironment

(Cooper *et al.* 1999), notably tumour necrosis factor-β and interleukin-1β, but not interleukin-6 or TGFβ. Furthermore, 11β-HSDs in bone demonstrate several unusual, 'unique' features. In contrast to other tissues, in bone 11β-HSD1 functions predominantly as a dehydrogenase, suggesting a 'protective' role in adult bone – thus the presence of these enzymes in osteoblasts may be an important determinant of glucocorticoid activity or inactivity (Fig. 2). The kinetic properties of these enzymes could also account for some of the biphasic responses seen with glucocorticoids. In addition, 11β-HSD2 in fetal bone co-localises with both glucocorticoid receptor and mineralocorticoid receptor, suggesting a possible role for glucocorticoid-mediated signalling via the latter receptor in normal bone physiology or development.

Summary

Glucocorticoids clearly regulate several crucial determinants of osteoblast differentiation, proliferation and function. These effects are complex and at times opposing, depending upon dose and stage of osteoblast differentiation. In addition to improving our understanding of glucocorticoid action, we are now increasingly aware of important factors that regulate glucocorticoid 'inactivity' within osteoblasts.

In keeping with other peripheral target tissues, the expression of 11β-HSD isozymes within human bone suggests that corticosteroid hormone action is modulated at a pre-receptor level. Further studies are required to define the regulation of 11β-HSD within human osteoblasts at varying stages of differentiation, but the manipulation of 11β-HSD within bone itself may, in the future, offer a novel therapeutic approach in modulating glucocorticoid 'activity' or 'inactivity' in bone.

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