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Review

Glucocorticoids and bone: cellular, metabolic and endocrine effects

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INTRODUCTION

Chronic exposure to excessive concentrations of endogenous cortisol or to pharmacologic doses of glucocorticoids (GCs) causes multiple deleterious effects on body structure and function. Osteopenia, osteoporosis and bone fractures are well recognized consequences of excessive GC exposure, with fractures being a major cause of morbidity and mortality, particularly in the elderly. In particular, GCs have profound effects on bone metabolism, acting at many sites. They increase bone resorption and can dramatically decrease bone formation. The mechanism of increased bone resorption has not been fully elucidated, and the mechanism of decreased bone formation is complex. Some of the derangements caused by chronic exposure to excessive GC concentrations are treatable and potentially reversible, depending on the extent of the damage.

Bone remodelling is regulated by systemic and local factors, and glucocorticoids exert a significant impact on the skeleton. Bone remodelling is tightly regulated, and bone formation occurs in areas of previously resorbed bone. Bone is continuously regenerated, a process that is undertaken by basic multicellular units. These units are comprised of teams of juxtaposed osteoclasts and osteoblasts. These bone-resorbing- and-forming cells maintain bone remodelling in an orderly fashion, and osteoclastogenesis is dependent on the genesis and presence of osteoblasts. The number of bone-forming and bone-resorbing cells present in the basic multicellular units is also dependent on an orderly cellular death or apoptosis. Therefore, cell genesis and death are critical for the maintenance of bone homeostasis. The genesis of osteoblasts and osteoclasts is governed by specific genes, local regulatory factors and various systemic hormones, including glucocorticoids[1].

Glucocorticoids regulate gene expression by transcriptional and posttranscriptional mechanisms. The transcriptional effects, which have been studied in greater detail, are mediated by the glucocorticoid receptors (GR) and occur by activation or repression of gene expression[2].

To differentiate between the various modes of GC action in development and physiology, mice carrying a DNA binding defective GR have been created[3,4]. In contrast to mutants with a disrupted GR gene, mice carrying a DNA binding defective GR are able to survive after birth, but *trans*-activation functions of the GR are absent. The model allows discrimination between DNA binding-dependent and independent functions of GR, responsible for a specific activity[3,4]. Another useful model is based on the use of targeted

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gene inactivation, which allows for the tissue-specific excision of a selected gene[5]. Bacteriophage P1 Cre recombinase is a 38 kDA protein that recognizes the 34-bp DNA sequence lox^P (locus of cross-over P1), and when lox^P sites flank a gene region, Cre induces an intramolecular recombination and excision of the intervening DNA[6]. This results in deletion of the DNA sequence flanked by lox^P. By placing the Cre recombinase under the control of a tissue-specific promoter, the function of the gene can be examined specifically in that tissue and can be inactivated at an appropriate time. For this purpose, transgenic mice overexpressing the Cre recombinase, under the control of a tissue-specific promoter, are mated with mice in which a specific gene is flanked by lox^P sequences. The progeny will carry the tissue-specific gene deletion. Using this model, the GR gene has been inactivated selectively in the liver, thymus, monocytes/macrophages and brain, respectively, but targeted inactivation in skeletal tissue has not been carried out[7]. This will be required to define the physiological role of glucocorticoids in bone. Levels of expression of the GR may modulate glucocorticoid action in bone, and selected cytokines, such as IL-6 and IL-11, respectively, increase and decrease GR levels, possibly sensitizing or desensitizing osteoblastic cells to the effects of glucocorticoids[8].

GCs and bone formation

GCs have profound effects on bone formation. An inhibitory effect is well documented in both humans and rats, and is presumably mediated by GR, which has been demonstrated in osteoblasts. The mechanism of the reduction in bone formation is complex. GCs have a direct inhibitory effect on osteoblasts[9], which is mediated by three actions: (a) inhibition of the replication of osteoblastic lineage; (b) a decrease in the genesis of new osteoblastic cells; and (c) induction of osteoblastic cell death and/or apoptosis. Even though glucocorticoids increase the apoptosis of fully formed mature osteoblasts, this is not the case in immature cells. This is in part because GCs have complex effects on osteoblast generation and death. For example, when primary rat osteoblasts are cultured under differentiating conditions in the absence of cortisol, the cells differentiate, mineralize and undergo apoptosis. The addition of cortisol results in a decreased cell differentiation and impaired maturation and mineralization. Consequently, the lack of terminal cell differentiation under these experimental conditions results in a decrease of cellular death[10]. The decrease in osteoblastic maturation is in accord with the inhibitory effects of glucocorticoids on the differentiated function of the osteoblasts[11]. Consequently, GCs can deplete the cell population capable of forming new bone. GCs also inhibit bone matrix synthesis by decreasing type 1 collagen synthesis and by modulating the expression of mRNA encoding osteopontin, fibronectin, β-integrin and bone sialoprotein[12]. Moreover, the mechanism of GC-induced bone damage also involves an indirect effect mediated by a number of local growth factors[13].

Recent attention has been focused on the effects of GCs in the cellular differentiation of osteoblasts toward adipocytes[14]. Although some investigators have reported that GCs may induce osteoblastic differentiation, this is inconsistent with the loss of cells of the osteoblastic lineage and of osteoblastic function observed after glucocorticoid exposure[15].

In fact, GCs seem capable of shifting the differentiation of stromal cells toward the adipocytic lineage. This shift may involve the regulation of nuclear factors of the CCAAT/enhancer binding protein (C/EBP) family and of peroxisome proliferators-activated receptor γ2 (PPARγ)[16]. Of the six C/EPBs identified, C/EBPα, β, and δ play essential roles in adipogenesis. Recently it was demonstrated that cortisol induces Notch1 mRNA levels in osteoblasts and Notch 1 plays a role in adipogenesis[13,17]. Notch consists of a family of four transmembrane receptors activated by their ligands Delta and Jagged. Notch 1 and 2 and their ligand, Delta 1 and Jagged 1, are expressed by osteoblasts. Overexpression of Notch 1, which is increased by cortisol, in stromal and osteoblastic cells mimics some the effects of glucocorticoids, impairing osteoblastic maturation and favoring adipogenesis[18-19].

GCs and bone resorption

Although the fundamental action of glucocorticoids in bone is mediated by a decrease in bone formation, in vivo findings in animals and humans are consistent with an early increase in bone resorption occurring after exposure to glucocorticoids. This is probably responsible for the rapid bone loss observed in humans after the initiation of glucocorticoid therapy and explains the effectiveness of antiresorbing agents in the management of glucocorticoid-induced osteoporosis[20].

The effects of GCs on bone resorption are still not clearly understood. In organ cultures, as well as in vivo, GCs have a wide spectrum of effects, depending on the experimental model used. It is hypothesized that GCs decrease bone resorption via an increase in the rate of receptor-mediated apoptosis of osteoclasts. In humans, some, although not all, histomorphometric studies have suggested an increase in bone resorption[21].

GCs increase the expression of receptor activator of NF-kappa B ligand (RANK-L) and decrease the expression of its soluble decoy receptor, osteoprotegerin, in stromal and osteoblastic cells[22]. GCs also enhance the expression of colony-stimulating factor (CSF)-1, which in the presence of RANK-L induces osteoclastogenesis[23]. These actions likely explain the increased bone resorption that follows skeletal exposure to glucocorticoids. Eventually, a more chronic state of decreased bone remodelling develops, which is secondary to a loss of cells signalling to osteoclasts or to their progenitors, and to the apoptosis of mature osteoclasts[24]. However, under selected experimental conditions, GCs were found to extend the life of the osteoclast and to oppose the effect of bisphosphonates on osteoclast apoptosis[25]. There is additional evidence for glucocorticoid effects on bone resorption, as these steroids enhance the expression of collagenase-3, a metalloprotease that plays a central role in bone resorption. The stimulatory effect of GCs on collagenase expression occurs in osteoblastic cells by a posttranscriptional mechanism[26].

Indirect actions of GCs on bone metabolism

1) GCs and intestinal Ca^{2+} absorption: It is generally accepted that GCs decrease net intestinal Ca^{2+} absorption in both humans and animals, but the mechanism is not known. Radioisotopic techniques have shown Ca^{2+} absorption to decrease, increase or remain unchanged. The GC effect on intestinal Ca^{2+} absorption depends on several factors, such as the experimental model, the intestinal segments and the dose of GC administered. In particular, in the duodenum GCs cause an inhibition of the active transcellular Ca^{2+} transport, a decrease in the synthesis of Ca^{2+}-binding protein and an increase in the rate of degradation of $1,25(OH)_2$ vitamin D at its mucosal binding site[27].

2) GCs and renal Ca^{2+} absorption: A sustained GC excess results in marked hypercalciuria, probably mediated at many sites depending on the timing of GCs administration. In fact, GCs might have a direct inhibitory effect on tubular reabsorption, particularly in short-term administration of high doses[27].

3) GCs and the somatotropic axis: Immunological studies have shown that total hypothalamic GHRH peptide content falls in glucocorticoid-treated rats compared with controls[28]. Similarly, Fernandez-Vasquez et al[29] reported that treating rat hypothalamic cells in vitro with high doses of corticosterone decreased neuronal GHRH release. Recent immunocytochemical data showed a reduction of optical density and percentage area of immunostaining for GHRH only in the rostral region of the median eminence of the hypothalamus in glucocorticoid exposed rats[30]. The effect of glucocorticoid treatment on the somatostatinergic system are tissue specific and apply to both somatostatin peptide and mRNA content[31]. The rise in hypothalamic somatostatin content due to glucocorticoids seems to reflect an increase in transcription of the somatostatin gene[30]. RNAse protection assay also revealed a second lower molecular weight somatostatin gene-transcription product in glucocorticoid-treated rats, suggesting possible control of somatostatin gene expression both quantitatively and qualitatively. In humans, the inhibitory effects of GCs on growth hormone (GH) secretion are predominant and probably dependent on an increase in somatostatin synthesis and secretion, which blocks pituitary GH secretion[32]. The GH response to GH-releasing hormone (GHRH) in normal humans after a single dose of cortisone acetate (50mg) is significantly reduced with respect to controls[32]. Numerous studies have examined GH secretion in prepubertal children undergoing long-term immunosuppressive glucocorticoid-treatment[33]. In these children, GH responses to various pharmacological stimuli are reduced, as expected from the adult paradigm. Similarly, spontaneous GH secretion is decreased[33]. Pyridostigmine significantly enhances both GHRH and sleep induced GH release in children, although partially, on long-term glucocorticoid treatment. The pyridostigmine effects are consistent with, but not proof of, somatostatin's involvement in glucocorticoid inhibition of GH secretion in children[34]. Indeed, pyridostimine's partial efficacy in chronic glucocorticoid thera-

py could also reflect restricted GHRH release and hence reduced stimulation of GH secretion by pyridostigmine. GH secretion in adults receiving chronic immunosuppressive therapy with glucocorticoids is also impaired. Pharmacological agents such as clonidine and galanin, which are presumed to affect GH release in part via increased hypothalamic GHRH release, are less effective in enhancing baseline GH concentrations and facilitating GHRH-induced GH release in glucocorticoid-suppressed adults compared with controls[35,36]. On the other hand, L-arginine, which acts as a so-called functional somatostatin antagonist[32], virtually normalizes the GH-secretory response to GHRH in adults treated with glucocorticoids[37,38]. The clinical significance of the suppressed GH/IGF-1 axis in adults treated with glucocorticoids is reinforced by short-term intervention trials. GH administration elicits a significant increase in nitrogen balance, serum osteocalcin, the carboxy-terminal propeptide of type I procollagen and carboxy-terminal telopeptide of type I collagen in patients receiving glucocorticoid treatment[39]. Indeed, glucocorticoid-induced protein catabolism is reversed during co-administration of GH[40], whereas co-treatment with IGF-1 and GH elicits net anabolism[41]. In patients undergoing long-term glucocorticoid therapy for non-endocrine diseases, GH co-administration is also able to significantly lower total and low-density lipoprotein cholesterol, but increases serum trygliceride levels[39].

4) Selected actions of GCs may also be secondary to a direct effect on IGF-1. IGF 1 has stimulatory effects on bone formation, opposite to those of glucocorticoids, and its skeletal levels are decreased by cortisol[42], which regulates the binding of C/EBPs to a recognition site adjacent to the third start site of transcription. This results in an inhibition of IGF-1 transcription[43]. GCs also regulate various IGF-binding proteins. In fact, they inhibit the transcription of IGF-binding protein-5, a binding protein reported to have stimulatory effects on bone formation[44].

5) GCs and the gonadotropic axis: Excessive GC exposure might inhibit the hypothalamic-pituitary-gonadal axis in both sexes, acting at different levels[45]. In fact, the effects of GCs on the gonadotropic axis might be dependent on: (a) a decrease in gonadotropin-releasing hormone (GnRH); (b) a reduction in the luteinizing hormone (LH) response to LH-releasing hormone (LHRH) in both men and women; (c) a reduction in the number of gonadotropin-binding sites in the ovary and the testis; and (d) peripheral inhibition of estrogen and testosterone production[20].

6) GCs and parathyroid hormone (PTH): Among the mechanisms by which GCs induce bone resorption, a hyperparathyroid state has been considered to be of some relevance. Earlier literature showed increases in serum levels of PTH when patients exposed to chronic glucocorticoids were studied[46,47]. Other hypotheses have included enhanced sensitivity to PTH due to changes in the number and affinity of PTH receptors[46]. However, acute and chronic use of glucocorticoids is not consistently associated with elevated endogenous levels of PTH[48]. Recent studies have established that in healthy young subjects, PTH secretion has two major components: a predominantly tonic pattern of constant secretion and low amplitude pulses with high frequency (approximately every 15-20 minutes). In healthy individuals, pulsatile PTH secretion accounts for approximately 25% of the total secreted PTH[49,50]. We have recently found that PTH secretory dynamics are altered by glucocorticoids with a reduction in the tonic component and an exaggeration in the pulsatile component of PTH secretion that override normal secretory dynamics[51]. These pulsatility studies examined the relationship between PTH and glucocorticoids in the context of PTH secretory dynamics, calling attention to the need to consider not only the amount of PTH secreted, but also to its pattern of secretion in the presence of glucocorticoids. The regulatory physiology by which glucocorticoids induce a redistribution of spontaneous PTH secretion is not known. Levels of vitamin D in the lower range of normal could conceivably be a secretory trigger. Finally, it is possible that glucocorticoids act directly at the parathyroid gland to affect PTH secretion. In this regard, glucocorticoids may have actions that govern more the secretory behaviour of PTH than the actual amount secreted over a period of time[52]. GCs might affect PTH secretion via two mechanisms: (a) a direct stimulation of PTH secretion as demonstrated in cultured parathyroid tissue; and (b) a reduction in intestinal absorption and an increase in urinary excretion of

Ca^{2+}. Altogether, the evidence has clearly shifted away from the notion of secondary hyperparathyroidism in GIO[48]. On the contrary, PTH secreting dynamics appear to play a significant role in the pathogenesis of glucocorticoid-induced osteoporosis.

7) GCs and vitamin D metabolism: The possible contribution of alterations in vitamin D metabolism to GC-induced change in CA^{2+}absorption have been studied extensively, with divergent findings. In GC-treated subjects, normal or low levels of 25-OH vitamin D have been documented, and these are probably the result of differences in dietary intake and absorption of vitamin D, and difference in sunlight exposure in the various populations studied. Concerning $1,25(OH)_2$ vitamin D, normal serum concentrations in adults and variable levels in children (an increase with short-term GC administration and a reduction with long term GC therapy), have been shown[20].

PERSPECTIVES AND CONCLUSIONS

It is well known that GC sensitivity (and, consequently, the side effects of this therapy, such as bone loss) might vary among individuals. The characterization of the individual's susceptibility to GC damage could represent the future preventive or therapeutic approach to GC-induced bone loss.

11βHSD1 is a low affinity NADP(H)-dependent enzyme, which displays primarily reductase activity and converts cortisone to cortisol[53]. 11βHSD1 acts as a pivotal determinant of steroid responses in bone, amplifying glucocorticoid signalling in osteoblasts[54]. At the clinical level, recent analysis of age-specific variations in osteoblastic 11βHSD1 activity suggests that this mechanism is a contributing factor in age-related and glucocorticoid-induced bone loss[55]. In addition, pro-inflammatory cytokines, often present in excess because of the underlying disease being treated with glucocorticoids, can modulate11βHSD1 and amplify the effect of steroids in bone[56].

In conclusion, based on these pathophysiological insights, a more individualized therapeutic approach can be used for osteoporosis in patients treated with GCs which, in combination with recently published guidelines, may comprise antiresorptive (bisphosphonates), anabolic (PTH) and hormonal (HRT, testosterone, GH) treatments[57,58].

Future research into the basic mechanism of glucocorticoid action in bone and on the use of selective glucocorticoid receptor modulators or of nitrosylated derivates of prednisolone may result in new approaches to the management of GIO[59]. Another important area of future research will be the mechanism of corticosteroid-induced myopathy, a serious complication that may lead to falls and fractures. Recent works demonstrating upregulation of myostatin, a negative regulator of muscle mass, by dexamethasone, offer new information on the possible mechanisms involved[60].

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