Stimulation of Sclerostin Expression by Glucocorticoid Hormones: A Mechanism to Explain Steroid-Induced Inhibition of Bone Formation

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Introduction

The osteopenia associated with glucocorticoid therapy is a serious side effect of steroid use. The mechanism by which this occurs has been reported to be due to an increase in bone resorption. However, a number of studies have also implicated a decrease in the coupled formation of bone by osteoblasts. The mechanisms by which this osteoblast activity is blunted by glucocorticoid hormones has been elusive to define.

Sclerostin is a product of the SOST gene and is known to be a potent inhibitor of osteoblast activity. Its mechanism of action is purported to be through inhibition of the Wnt/beta-catenin signaling pathway by virtue of its ability to block Wnt binding to the receptors, LRP5/6 and Frizzled. Sclerostin was first identified as a factor produced by osteocytes, however, more recent reports show that a number of cell types can produce the molecule, including osteoblasts, chondrocytes, kidney cells and smooth muscle cells in blood vessels. SOST gene expression is tightly regulated in all of these cell types.

In this study we posed the question, is SOST gene expression regulated by glucocorticoid hormones and could this regulation contribute to the inhibition of bone formation seen in patients on steroid therapy?

Methods

For investigation of the SOST promoter sequences we utilized the search programs of the Transcriptional Regulatory Element Database of Cold Spring Harbor Laboratory.

For the in vitro cell experiments we used osteoblasts isolated from neo-natal rat calvaria. Periosteal calvarial explants from 2-3 day old rat pups were sequentially digested with a collagenase preparation. We collected and used the cell fractions representing progenitor and mature osteoblasts. Cells were treated with dexamethasone over a range of concentrations and times of exposure.

Analysis of SOST gene expression was performed with RT-PCR for sclerostin mRNA and with Western immunoblotting for sclerostin protein.

Results

An analysis of approximately 5.5 kB of promoter sequence up-stream of the transcriptional start site of the SOST gene revealed a number (at least 4) of glucocorticoid response elements (GRE’s) clustered between -4.7 and -5.3 kB. This finding suggests a possible regulation of SOST gene expression by glucocorticoid hormones. To directly investigate this we measured sclerostin mRNA and protein levels in osteoblasts exposed to dexamethasone.

Figure 1 shows that osteoblasts exposed to dexamethasone over a range of concentrations showed an apparent biphasic stimulation of sclerostin mRNA production after a six hour treatment. Maximum stimulation was approximately 3.5 fold over vehicle treated cells at a concentration of 1.0 micromolar. At 5.0 micromolar, the effect appeared to diminish. At longer times of exposure (12 and 24 hours) there was still a stimulation in mRNA levels, however, the effect was more variable (data not shown).

Discussion

Although there are likely to be a number of mechanisms by which glucocorticoid hormones (i.e. dexamethasone) depress osteoblast function, it appears that elevation of sclerostin expression may be one of them. And given that the effects of sclerostin are extremely potent, its elevation by dexamethasone may explain much of the effect seen in animal models and humans. If further investigations support this finding it would suggest that anti-sclerostin therapy in the treatment of glucocorticoid-induced osteoporosis could be a particularly effective approach.

Significance

Glucocorticoid hormones can cause a rapid loss of skeletal mass. Both bone resorption and bone formation are targeted by these hormones. Our data suggest that the effect of the hormones on bone formation is mediated, in part, by a strong up-regulation of the potent osteoblast inhibitor, sclerostin. This finding implies that blocking sclerostin action in steroid-treated patients may be very effective in managing their osteoporosis.