GSK-3 Inhibitor BIO Stimulates Osteoblast Differentiation and Bone Formation

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Introduction: BIO (6-biomoinidirubin-3’-oxime) is a shellfish-derivative that is the most selective cell-permeable pharmacological inhibitor of GSK-3 (1, 2). In the absence of the Wnt signal, GSK-3 inhibits Wnt/beta-catenin signaling by promoting beta-catenin phosphorylation and subsequent proteasome degradation. In the presence of the Wnt signal, GS3-3 is inactivated and beta-catenin is free to accumulate and translocate into the nucleus to activate downstream target genes. BIO is the only known GSK-3 inhibitor that mimics Wnt/beta-catenin signaling and stimulates beta-catenin accumulation.

Beta-catenin promotes osteoblast-mediated bone formation. Inactivating mutation in the Wnt receptor, LRP5, results in a decrease in osteoblast numbers and bone mass in both humans and mice. Furthermore, mice lacking Axin2, an inhibitor of Wnt/beta-catenin signaling, display a craniosynostosis-like phenotype as well as increased osteoblast proliferation and differentiation. These findings support the critical role of beta-catenin in osteoblast differentiation and mark beta-catenin as a possible target for anabolic therapy against osteoporosis.

Given the ability of BIO to enhance Wnt/beta-catenin signaling and the effect of beta-catenin on modulating osteoblast activity, we explored the effects of BIO on osteoblasts. Consistent with findings in other cell types, our experiments reveal that BIO mimics the Wnt/beta-catenin signaling in osteoblasts. Moreover, BIO promotes osteoblast differentiation in vitro and bone formation in vivo.

Materials and Methods: Primary calvaria osteoblast isolation
Primary mouse osteoblast precursors were isolated from P1 mouse calvaria as described (3). Isolated cells were cultured in α-MEM media containing 10% fetal calf serum. Only the first passage cells were used. Primary osteoblasts were seeded in 12 well plates for 48 hours. To evaluate changes in osteoblast differentiation, primary osteoblasts were cultured in differentiation media containing 50 μM/μl ascorbic acid and 4 mM β-glycerophosphate.

Local injection over mouse calvaria
Male CD1 mice, aged 4 weeks, were obtained from Jackson Laboratories. BIO was injected subcutaneously over the right side of calvaria of mice. BIO was injected for 5 days in 50 μl of corn oil. FGF-1 was used as a positive control and was injected as described (4). 21 days after the last injection, the mice were anesthetized and euthanized for histology analysis.

Histology
Mouse calvaria were fixed for 48 hours in Formalin and were decalcified in EDTA for 3 weeks. The samples were then embedded in paraffin, cut in cross-section at 5 mm thickness, and H&E Orange-G staining and TRAP staining was performed as described (4).

Results: 1. BIO mimics Wnt/beta-catenin signaling in primary mouse osteoblasts. Primary calvarial osteoblasts, isolated from beta-catenin signaling reporter (TOP-gal) mice, were treated with BIO (1 mM) and Wnt3a (300 ng/ml). Compared to untreated controls, both BIO and Wnt3a treated cells showed a four-fold induction in the reporter activity as measured by β-Gal activity. Consistent with this finding, over a 12-hour time course, BIO up-regulated the level of active (non-phosphorylated) beta-catenin within 2 hours, peaking at four hours, as measured by Western blot. Similar to the Wnt signal, BIO also induced nuclear beta-catenin translocation and accumulation in primary osteoblasts as observed using a fluorescent-labeled anti-beta-catenin antibody.

2. BIO promotes osteoblast differentiation in vitro. Primary osteoblasts treated with BIO for 0, 2, and 4 days showed a four-fold increase in alkaline phosphatase (ALP) activity when compared to untreated cells. Quantitative PCR analysis revealed increased expression levels of Runx2 and Osteocalcin (OC), two osteoblast differentiation marker genes. Osteoblasts treated with BIO also show a dose-dependent increase in nodule formation and calcified matrix deposition in culture. These findings demonstrate that BIO stimulates osteoblast differentiation in vitro.

3. BIO induces bone formation in vivo. BIO was injected locally into the subcutaneous tissue of the mouse calvaria at 25 and 50 μg/day/mouse for 5 days. Vehicle injection was used for baseline measurements. H&E Orange-G staining and calcein double labeling showed that BIO induced new woven bone formation in mouse calvaria at both 25 and 50 μg doses (3 and 4-fold increase, respectively).

4. BIO does not regulate osteoclasts in vivo or in vitro. A potential mechanism for BIO-induced bone formation could lie in the ability of Wnt/beta-catenin signaling to regulate OPG expression and inhibit osteoclast activity. To determine whether BIO has an effect on osteoclastogenesis, we performed TRAP staining and counted osteoclasts using the calvarial samples derived from BIO-treated mice. There were no significant changes in osteoclast numbers in BIO-treated mice. In addition, splenocytes, treated with BIO, did not display differences in osteoclast formation. Finally, quantitative PCR analysis did not show differences in OPG expression. These findings suggest that the effect of BIO on bone is osteoblast-specific.

Discussion: The in vitro and in vivo results presented in the present study support the hypothesis that the GSK-3 inhibitor, BIO, has anabolic effects on bone formation. These effects are osteoblast-specific and mimic Wnt/beta-catenin signaling in osteoblasts.

Current treatments for osteoporosis, which affects over 10 million Americans over age 50, revolve around inhibiting catabolic events in bone. Given that osteoporosis is believed to be due to an imbalance in bone resorption and deposition, it is likely that in addition to anti-catabolic agents, anabolic treatments could greatly benefit patients with osteoporosis by restoring balance between these two processes.

Our findings suggest that GSK-3 inhibitors are promising tools in the therapeutic treatment of osteoporosis. Besides its effects on enhancing osteoblast differentiation and bone formation demonstrated in this study, BIO has been shown to be one of the few GSK-3 inhibitors that is selective and has an IC50 in the low nanomolar range. Future studies are needed to further characterize the effects of GSK-3 inhibitors on bone and their possible role in the treatment of osteoporosis.

References:

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