

Bone Safety – the Problem and the Solution

Christopher Jerome, B.Vet.Med., Ph.D.
President, Think Bone Consulting, Inc.
chris.jerome@thinkboneconsulting.com

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Summary

The Problem

Off-target effects in bone are being identified with increasing frequency, but standard preclinical toxicology study designs are not particularly sensitive to, and do not allow for thorough characterization of, skeletal effects. Consequently, development programs may be delayed due to late detection of, or the need for follow-up studies to characterize those effects. Recent examples of off-target skeletal effects are highlighted and a brief review of drug classes likely to trigger them is also included.

The Solution

Simple and cost-effective methods to improve detection and characterization of bone effects in preclinical studies are briefly described.

The Problem

Increasingly, Think Bone Consulting is contacted by clients dealing with off-target effects in bone or cartilage, rather than questions about efficacy evaluation of drugs that target bone. A typical circumstance is that a standard toxicology study finds histopathological evidence of bone changes such as increased osteoclast/osteoblast numbers on bone surfaces, or increased/decreased thickness of trabeculae.

Unfortunately, standard toxicology study designs often do not include collection and processing of samples that would be useful to further elucidate such findings, meaning that expensive and time-consuming follow-up studies are required. There is also a risk that standard studies are relatively insensitive to biologically significant bone and cartilage effects, which may therefore only be revealed in longer term preclinical or other studies performed relatively late in development.

The purpose of this paper is first, to summarize recent experience and literature suggesting that off-target bone effects can be expected in a broad array of drug development programs, and second, to propose proactive ways to deal with the potential for off-target bone effects.

Off-Target Effects on Bone

Specific Examples

SGLT inhibitors: A number of companies are actively developing SGLT2 inhibitors for the treatment of diabetes. These agents inhibit the re-uptake of glucose in the renal tubules, and thereby help to control blood sugar. Off-target observations include increased bone mass, decreased bone biomarkers, and decreased circulating levels of PTH and vitamin D metabolites

[12]. The data are consistent with an increase in intestinal calcium absorption, perhaps due to increased glucose concentration secondary to cross-reactivity with SGLT1 receptors in the gut. A similar mechanism for increased calcium absorption was previously described [3].

Antidiabetic thiazolidinediones: As is now well known, the antidiabetic drugs pioglitazone and rosiglitazone are clinically associated with decreased bone mass, effects on bone biomarkers, and increased rates of fracture [14]. Adverse effects of these agents on bone in animal models only became apparent several years after their introduction into clinical use [17].

Anti-retroviral drug: Offspring of pregnant monkeys treated with tenofovir were found to have skeletal abnormalities similar to rickets [18]; subsequently newborn and infant monkeys treated long-term with high doses were also found to have skeletal abnormalities and the lesions were linked to high-dose renal toxicity causing phosphate wasting [4,20]. This example highlights the potential for fetal effects in reproductive toxicology studies.

Important Drug Targets with Known Bone Effects

New therapies increasingly target pathways that are common to many tissues, including bone and cartilage. For example:

- The **MAPK/ERK pathway** is a common target for oncology drugs, and when targeted has been shown to have bone effects *in vitro* [10] and *in vivo* [13].
- **cFMS kinase** inhibitors, candidates for autoimmune disease and cancer therapies, similarly have bone effects in a number of model systems [5].
- Inhibition of the **mTOR pathway** yields immunosuppressant and antiproliferative activities that are clinically important to prevent transplant rejection and may yield oncology therapies. The prototypical pathway inhibitor rapamycin has effects on growth plate and endochondral bone growth [1,18], and on fracture repair [11].

Furthermore, in addition to the usual suspects such as sex steroids and glucocorticoids, an array of more specific current drug targets have been linked to skeletal effects:

- **Psychotropic drugs** [21].
- Selective **thyroid hormone receptor agonists** are currently being studied for weight and cholesterol reduction [7,9], but thyroid hormone is known to have effects on both mature and immature skeletons [2,8].
- **Acid suppressing drugs** such as proton pump inhibitors and H₂ receptor antagonists [6,22].

A recent review of drug-induced osteoporosis lists several classes of compounds clinically associated with osteoporosis [15], and the above overview suggests there is good reason to expect more examples to arise in the coming years. It seems prudent therefore to consider ways in which detection and characterization of off-target effects in the skeletal system might be improved.

The Solution

Assessment of Bone in Preclinical Studies – General Principles

Standard assessments of bone in toxicology studies consist of gross examination of several bones and joints, and histopathological examination of one or two bones. Gross abnormalities of bones and joints are rare, and while histopathological examination may detect some biologically significant changes in bone mass and bone remodeling, there are simple and cost-effective

techniques that would both detect a broader array of potentially significant changes and provide better characterization of those changes.

- Toxicology studies are usually performed in young, growing animals. Most agents that affect bone have more readily detectable effects in growing bones than in adult bone, due to both the sensitivity of growth processes and the high rate of bone remodeling activity that accompanies growth. Simple evaluations of bone size, weight, and gross morphology should therefore be sensitive to such effects.
- Biologically significant changes in bone mass and quality cannot occur without changes in the processes that shape the bone and determine its mass and composition: bone resorption and formation. Serum and urine biomarkers of these processes are readily available for laboratory species and can be used for screening, or run on stored samples if indicated by other data.
- Many bone abnormalities are accompanied by changes in bone mineral content, which are quantifiable by a variety of x-ray based methods. These methods can be applied to routinely fixed specimens, preferably of whole bones.
- Standardized procedures for bone histomorphometry have been in use for at least two decades [16], and are more sensitive to bone changes than standard histopathological evaluation. However, successful use of these methods depends on standardization and control of site and plane of section.

Specific Recommendations:

- Collect and store serum and urine aliquots for possible bone biomarker evaluations during the course of toxicology or pharmacokinetic studies. Most bone biomarkers are stable in samples stored at -70°C. Consider analyzing a few key biomarkers as part of such studies.
- Collect femurs and/or tibias, clean of soft tissue, record fresh weight, and fix intact. Standard 10% NBF is fine for most purposes. These specimens can be used for radiography, bone densitometry, μ CT imaging, or bone histomorphometry if indicated.
- Radiograph the femur and/or tibia using standardized positioning, preferably in the presence of a density standard such as an aluminum step wedge.
 - Qualitatively evaluate the radiographs.
 - Measure bone length on the radiographs (or on fresh/fixed bones) between clearly identifiable anatomical landmarks.
 - Perform radiographic densitometry.
- Develop SOPs for standardizing location and plane of bone sections, and ensure that articular cartilage, growth plate and metaphyseal spongiosa are included in standard sections. Such sections will then be amenable to histomorphometric analysis should it be indicated by other bone findings.

Your Solution: Think Bone Consulting

Think Bone Consulting, Inc. can provide assistance at all levels of bone safety evaluation:

Advice: Based on many years of experience in bone biology, pharmacology, and pathology, Think Bone can advise on preclinical study design and methods, and assist with interpretation of biomarker and morphological data on skeletal effects of drugs and devices.

Services:

- Radiography and radiodensitometry.
- Bone histology and bone/joint histomorphometry and grading services.
- GLP validation of bone histomorphometry systems.
- Custom development of image analysis routines in ImageJ.

Supplies: Think Bone is now offering aluminum and acrylic step wedges for standardization and calibration of radiographs. Coming soon: hydroxyapatite step wedges and a rotating slide holder for standard microscope stages that will make histomorphometric analysis so much easier!

Further information:

chris.jerome@thinkboneconsulting.com | +1 (360) 221-6723 | www.thinkboneconsulting.com

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