BoneKEy-Osteovision. 2007 March;4(3):94-98
http://www.bonekey-ibms.org/cgi/content/full/ibmske;4/3/94
DOI: 10.1138/20070253

NEWS

All That Glitazones Is Not Gold

Popular medications used to treat type 2 diabetes appear to increase fracture risk

Neil A. Andrews

Managing Editor, BoneKEy

New analyses of clinical trial data by GlaxoSmithKline and Takeda Pharmaceuticals are now providing compelling evidence that thiazolidinediones (TZDs, also known as glitazones), which raise tissue sensitivity to insulin, increase fracture incidence in women taking these medications for the treatment of type 2 diabetes. Specifically, the results show that diabetic women, though not men, taking either rosiglitazone or pioglitazone, the two TZDs currently approved for the treatment of type 2 diabetes, are at an increased risk of experiencing fractures of the upper and lower limbs, including foot, hand and upper arm fractures. The new findings, though, are hardly surprising to bone researchers who have been examining the impact on bone of rosiglitazone and other so-called PPARγ agonists that work by activating peroxisome proliferator activated receptor-γ, a receptor residing inside the cell nucleus that controls gene transcription.

"We've been expecting this kind of result with rosiglitazone for quite a while," says Beata Lecka-Czernik, an associate professor at the Reynolds Institute on Aging at the University of Arkansas for Medical Sciences (UAMS) whose laboratory work has demonstrated that rosiglitazone causes bone loss in mice. "I've been convinced for a long time that rosiglitazone increases fracture risk," agrees Larry Suva, a professor of orthopaedic surgery at UAMS who has examined the impact of TZDs on bone along with Dr. Lecka-Czernik. Researchers were more curious to learn whether pioglitazone would also increase fracture incidence because there are relatively fewer studies of that particular TZD in the published literature.

However, while bone scientists tend to agree that the new results with rosiglitazone are a logical outcome of the basic research on TZDs and bone that has taken place over the past five years, they also are of the same mind that the current clinical data, if confirmed by more carefully designed clinical trials, raise questions that the basic research cannot yet answer. For instance, why did the increase in fractures occur in the upper and lower limbs, and not in the sites, like the hip and spine, traditionally associated with post-menopausal osteoporosis? TZDs are now being prescribed more frequently for younger patients — what impact will the agents have on the long-term skeletal health of these younger people? Why did women experience more fractures, but not men? While the basic science may have forecasted an increase in fracture risk, as yet it is unable to explain these specific new findings.

The Key Clinical Data

In February, GlaxoSmithKline issued a letter to health care providers notifying them of an increased incidence of fractures amongst women participating in a clinical trial, known as ADOPT, designed to assess how well rosiglitazone controlled blood sugar levels in comparison to two other medications, metformin and glyburide. In this trial, significantly more women taking rosiglitazone experienced fractures, particularly in the upper arm, hand and foot, compared to women taking metformin or glyburide. In its letter, the company noted that data consistent with the results from ADOPT have also emerged from another ongoing clinical trial of rosiglitazone in

BoneKEy-Osteovision. 2007 March;4(3):94-98
http://www.bonekey-ibms.org/cgi/content/full/ibmske;4/3/94
DOI: 10.1138/20070253

patients with type 2 diabetes designed to examine cardiovascular endpoints. In view of these findings, the FDA has released a MedWatch for rosiglitazone.

The results from ADOPT follow two other clinical studies that have examined the impact of TZDs on bone. The first, an observational study published last year that examined data from the Health, Aging and Body Composition (Health ABC) study, found an association between TZD use (rosiglitazone, pioglitazone, and troglitazone) and increased bone loss in 70-79 year-old diabetic women, though not in men. The second, a small, 14 week, randomized, placebo-controlled study in New Zealand, found a significant reduction in markers of bone formation and a significant decrease in bone mineral density at the hip in healthy postmenopausal women taking rosiglitazone, compared to women taking a placebo.

When considered together, the results from ADOPT and the New Zealand study strongly suggest that rosiglitazone causes bone loss leading to more fractures, according to Ann Schwartz, lead author of the study based on the Health ABC data. "Health ABC alone raised some concern, and we concluded that paper saying there should be clinical trials, but it was still an observational study," says Dr. Schwartz, an assistant professor in the department of epidemiology and biostatistics at the University of California, San Francisco. "The ADOPT trial compared diabetes therapies, the outcome was time to monotherapy failure, and fractures were recorded just as adverse events. However, the increased fracture risk was probably not just a chance finding given the results of the New Zealand trial. Its main outcome was bone markers, DXA measurements were collected, and the authors observed a pretty dramatic drop in bone density. That answers some of the questions."

Coming on the heels of the data from GlaxoSmithKline, Takeda Pharmaceuticals, after examining its clinical trial database, issued its own letter to healthcare providers at the beginning of March, finding a similar increased risk of upper and lower limb fractures in women (though, again, not in men) taking pioglitazone compared to women taking an active or placebo comparator. As with rosiglitazone, the FDA has issued a MedWatch for pioglitazone. That pioglitazone, in addition to rosiglitazone, increases fracture risk raises concerns that TZDs, as a class of medications, may have this effect.

Despite the recent findings from the pharmaceutical companies, experts agree that carefully-designed, prospective, randomized clinical trials, in diabetics or insulin-resistant individuals, specifically constructed to measure the impact of TZDs on bone are important to determine if some of the recent results are simply artifacts of study design. For instance, while the companies did not find an increased fracture rate at the spine, these fractures are difficult to identify, and the studies were not originally set up to find them, thus making firm conclusions impossible. "Half of spine fractures are asymptomatic, and only picked up by X-ray, so that's a big limitation of these studies," says Clifford Rosen, a physician at St. Joseph's Hospital and a senior staff scientist at the Jackson Laboratory in Maine who has submitted a grant proposal to the NIH for a randomized, placebo-controlled study of rosiglitazone in insulin-resistant individuals. "We don't know if these are truly atypical sites of fractures, or just the tip of the iceberg and that eventually we'll see more morphometric vertebral fractures."

In addition, it is possible that, over time, with a longer duration of TZD use, fractures will ultimately show up at additional sites, a possibility perhaps made more likely considering that the Health ABC and New Zealand studies did in fact find changes in bone density at the hip and spine. Furthermore, exactly how much bone loss glitazones cause, and how long the effect of the drugs lasts, remain unknown, according to Andrew Grey, lead author of the New Zealand trial and an associate professor of medicine at the University of Auckland in New Zealand. "We don't know how much bone is lost in either healthy or type 2 diabetic populations that are starting rosiglitazone and how profound the effects are on bone turnover with longer-term

treatment. We also don't know whether the effects are reversible: if the treatment is stopped, does the effect wear off?"

Nevertheless, experts agree that despite these limitations, when all the clinical studies are considered together, they point to the same general conclusion: TZDs have adverse skeletal effects in humans.

The Basic Science That Led the Way

Bone researchers who have studied the impact of rosiglitazone and other TZDs in the laboratory aren't surprised by this outcome, since their research has been pointing strongly in this direction for several years. They knew that osteoblast precursors, known as mesenchymal stem cells, have the potential to become either osteoblasts or adipocytes; PPARγ, an adipocyte master gene, can divert the stem cells from the osteoblast pathway into the adipocyte pathway when activated in the bone marrow. These facts, which accounted for the commonly accepted observation made many years ago that bone marrow becomes increasingly fatty, with decreased osteoblastogenic potential, with age served as the foundation of more recent *in vitro* work showing that rosiglitazone stimulates adipogenesis, and inhibits osteoblastogenesis, when added to bone marrow cultures.

"This led us to think that, with advancing age, PPARγ activation would cause a type of age-related bone loss," says Robert Jilka, a professor of medicine at UAMS and a VA research career scientist in Little Rock whose *in vitro* and *in vivo* studies, along with those by Dr. Suva, Dr. Lecka-Czernik, and others, are among the pioneering studies in the field. Indeed, studies published in 2004 demonstrated *in vivo* what the *in vitro* data had suggested: mice fed rosiglitazone exhibited decreases in osteoblastogenesis and bone formation, leading to bone loss. Consistent with this idea, another 2004 study found that mutant mice with only one working copy of the PPARγ gene exhibited high bone mass. The molecular mechanisms by which bone loss occurs as a result of rosiglitazone treatment are thought to include effects on the expression of osteoblast transcription factors like Runx2 and Osterix, as well as effects on cytokines and growth factors in the bone marrow micro-environment. There is also some evidence to suggest that rosiglitazone may have effects on cell survival.

The nature of the bone loss observed by the researchers in the early studies, though, as well as the short period of time that rosiglitazone had actually been used to treat people with diabetes, meant that effects on humans wouldn't be immediately apparent. "At the time, rosiglitazone had been on the market for only a few years," Dr. Lecka-Czernik explains. "We knew that the mechanism by which animals lost bone through activation of PPARγ did not result in acute bone loss, but rather in accumulated bone loss, so we knew that in humans we would need to wait several years to see the effect on bone."

Research Challenges

Now that enough time has elapsed and the clinical studies are demonstrating adverse consequences of TDZ treatment on bone, the basic research must now focus on a new set of questions. One of the most interesting ones concerns the site of the fractures identified in the drug companies' analyses. The data show an increased fracture incidence at the limb extremities, rather than at the more common sites of osteoporotic fractures, such as the hip and spine. Why did diabetics experience fractures at these unusual locations? Since the data from the pharmaceutical companies include younger women — the average age of subjects in ADOPT reached only the mid 50s — one possible explanation for the findings is that they simply reflect the kinds of fractures that younger women would tend to experience. However, it should be noted that the pharmaceutical companies did not break down their fracture data specifically by age.

Another possibility is that something specific to the pathology of diabetes is a contributing factor. "In diabetes, there is a combination of different factors: elevated glucose levels, which may affect the bone structure by itself, and changes in the vasculature of the lower extremities, which may also affect bone

BoneKEy-Osteovision. 2007 March;4(3):94-98
http://www.bonekey-ibms.org/cgi/content/full/ibmske;4/3/94
DOI: 10.1138/20070253

structure or bone quality," says Dr. Lecka-Czernik, who is also the guest editor of a recent special issue of *PPAR Research* on PPARs and bone metabolism.

This issue draws attention to a current dearth of knowledge on the bone physiology of type 2 diabetics. "All of our work in animal models, of course, is in normal mice, not diabetic mice," says Dr. Jilka. "The mechanism of bone loss may be different in diabetics, and if it's true that rosiglitazone does induce bone loss in diabetics and cause fractures, we need to know more about the metabolic state of the bone to begin with," he stresses.

Part of the reason why the bone field cannot yet give a convincing explanation of the unusual fracture sites is that its understanding of the mechanisms by which rosiglitazone acts to cause bone loss even in normal animals is limited, and emerging research is already beginning to paint a complicated picture. For instance, a new study by Dr. Lecka-Czernik, Dr. Suva and colleagues comparing the effects of rosiglitazone in normal mice at different ages suggests that the mechanisms involved may be age-dependent. "In adult animals, it looks like the effects of rosiglitazone are associated with osteoblasts, while in aged animals, it appears that the effects are associated with osteoclasts. Although the outcome — bone loss — is the same, the mechanism of the effect is different," says Dr. Suva. This finding presents an interesting potential therapeutic avenue. "The direction I think the field should go in," Dr. Suva continues, "is to look at the interactions of rosiglitazone and its accompanying molecules in the presence or absence of anti-resorptive agents, and in the presence or absence of anabolic agents, and to see if there is any opportunity for efficacy in treating diabetes and alleviating the effects of the drug on the skeleton."

The new study, published ahead of print on March 1 in *Endocrinology*, could turn out to be particularly relevant since increasing numbers of younger patients are being considered for treatment with TZDs, yet almost nothing is known about how this agent might affect the young, growing skeleton. "The big issue is, if teenagers are receiving prophylactic rosiglitazone because they are overweight and insulin-resistant, and this will prevent the onset of diabetes, then this possibly affects their later risk of fracture because the treatment might impair the acquisition of peak bone mass," Dr. Rosen says.

Even though the researchers discovered that aging was a confounding factor for bone loss induced by rosiglitazone that correlated with increased PPARγ expression in bone marrow stem cells, they also found that the skeletons of young, growing (1 month old) mice were still affected. "While we did not see a significant decrease in bone mineral density in young mice, we did see some significant changes in the bone formation rate," Dr. Lecka-Czernik explains. "These changes are probably masked by the relatively vigorously growing skeleton, but in the long run the skeleton may be weaker or have some other abnormality."

In addition to explaining the unusual fracture sites observed in diabetic patients thus far, a better understanding of the mechanisms of rosiglitazone-induced bone loss in normal animals may eventually help to account for another conundrum: why have all the clinical studies to date revealed an increased fracture risk in women, but not in men? It could be that the studies did not include enough men, or that, with longer use of rosiglitazone, more men will eventually experience fractures. On the other hand, the gender differences may be real, perhaps, Dr. Lecka-Czernik suggests, involving cross-talk between the estrogen and PPARγ receptors.

Treating Diabetes While Being Kind to Bone

Despite such uncertainties, TZDs do appear to be bad for bone. A hope exists, though, for the development and eventual clinical use of a PPARγ agonist that helps diabetics without adversely impacting the skeleton. "That's the holy grail," says Dr. Suva. "It's clearly within the realm of possibility that one could have an anti-diabetic, selective modulator that would be safe for the

BoneKEy-Osteovision. 2007 March;4(3):94-98
http://www.bonekey-ibms.org/cgi/content/full/ibmske;4/3/94
DOI: 10.1138/20070253

skeleton and wouldn't cause a loss of bone, but would still have the desired efficacy in the anti-diabetic arena."

Optimism for this view comes from studies showing that the reciprocal relationship between adipogenesis and osteoblastogenesis usually seen with PPARγ activation does not always apply. For instance, a 2002 study by Dr. Jilka, Dr. Lecka-Czernik and colleagues used a variety of oxidized fatty acids as ligands that bind to PPARγ to show that activation of the receptor need not always impact both fat cell and bone cell production. "Depending on which ligand for PPARγ was added, we saw every combination: some ligands only stimulated adipogenesis and didn't do anything to osteoblasts, some inhibited osteoblastogenesis but didn't touch adipocytes, and some, like rosiglitazone, did both," Dr. Jilka says.

While neither rosiglitazone nor pioglitazone resulted in a separation of the pro-adipocytic and anti-osteoblastic activities of PPARγ, a 2006 study by Dr. Lecka-Czernik, Dr. Suva and colleagues found that a synthetic TZD called netoglitazone did in fact produce this divergence. "We found that netoglitazone, although very efficient in lowering blood glucose levels at the doses we used, did not cause bone loss," Dr. Lecka-Czernik says.

While the ability of PPARγ to bind to many different ligands — it binds to so many, in fact, that it is known as a "promiscuous" nuclear hormone receptor — makes developing a selective PPARγ agonist good for diabetes and kind to the skeleton theoretically possible, that very promiscuity also poses significant real-world hurdles. Indeed, as Dr. Rosen notes, after binding to the receptor, rosiglitazone recruits numerous co-activators that ultimately determine which genes will be turned on or off, and the key will be understanding which co-activators particular ligands recruit or how they recruit them. A study of the genetic differences in the PPARγ gene related to bone density may make this understanding possible. "These polymorphisms could have a profound effect on whether the ligand suppresses bone formation or just increases marrow adiposity and doesn't affect bone," says Dr. Rosen, who is studying polymorphic differences both in congenic mice with overactive PPARγ activity, as well as in people. Dr. Rosen is also testing whether simply using lower doses of rosiglitazone will help diabetics without detrimental skeletal effects.

As this work unfolds, what do the new clinical findings mean for doctors and patients right now? The consensus is that caution, rather than alarm, is the wisest course, but doctors should be aware of the potential skeletal consequences of TZDs, particularly in women over the age of 50 and in those at the greatest risk of fracture. "The important thing is not to panic," says Nelson Watts, a professor of medicine at the University of Cincinnati College of Medicine who has written about TZDs and fracture risk. "This isn't telling us that we should avoid glitazones, but simply that skeletal factors should be considered in deciding which drugs to use in patients with diabetes." Ultimately, physicians will have to balance the skeletal risks of glitazones with the undeniable benefits they have brought to diabetic patients, and consider ways to mitigate those risks, particularly in the highest-risk patients.