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Should biochemical markers of bone turnover be considered standard practice for safety pharmacology?

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Abstract

The success in biomedical sciences such as genomics and proteomics is not paralleled in the medical product development methods. The consequence of this is a lack of translation into improved drug safety and efficacy. Therefore the US Food and Drug Administration (FDA) introduced the Critical Path Initiative in 2004 to modernize drug development and safety pharmacology. Bone is that largest tissue by weight, and is continuously remodelled. Changes in bone turnover lead to complications such as osteoporosis and fracture, that is associated with an increased mortality. Recent findings have identified bone as a possible endocrine organ and the availability of valid biochemical bone markers suggests that assessing bone turnover should also play an important role in general safety pharmacology.

Keywords: *Bone markers; safety pharmacology; Critical Path Initiative*

Introduction

Despite extensive progress in biomedical scientific technology and rising investment in pharmaceutical research and development, successful introduction of safe new drugs has experienced a downward trend in the last two decades (Woodcock & Woosley 2008). This trend is worldwide, and 2004 represented a low in the introduction of new molecular entities (NME), and the cost per successful NME has increased to an estimated $800 million or even more (Adams & Brantner 2006, DiMasi et al. 2003). It has been estimated that NMEs entering Phase I development have an 8% chance of reaching the market versus 14% chance 15 years ago, and the Phase III failure rate is now reported to be approximately 50%, compared to 20% 10 years ago (O'Neill 2006).

The success in biomedical sciences such as genomics and proteomics is not paralleled in the applied translational sciences and medical product development methods at a time when expectations about drug safety and efficacy are rising. The US Food and Drug Administration (FDA), having the simultaneous mission to protect and promote the health of the public recognized that its requirements for safety using last century's assessment methods are stifling innovation, and thus launched the Critical Path Initiative in March 2004. The Critical Path Initiative entailed a report titled 'Innovation or Stagnation? – Challenges and Opportunity on the Critical Path to New Medical Products' (http://www.fda.gov/oc/initiatives/criticalpath/whitepaper.html). The initiative is broad in scope and intends to engage stakeholders in academia, funding agencies and industry to facilitate and improve medical product development from candidate selection (safety and efficacy) to product launch and production. In March 2006 The FDA published the 'Critical Path Opportunities Report' after extensive consultations with stakeholders and FDA scientists (http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/CriticalPathOpportunitiesReports/default.htm). In this report, the development of biomarkers was identified as the highest priority for scientific effort. A year earlier the Critical Path Institute (C-Path, www.c-path.org) was founded by the University of Arizona and the FDA as a non-profit organization to act as a third party to support the FDA Critical Path Initiative and its fundamental role

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in creating and leading productive consortia such as the Predictive Safety Testing Consortium (PSTC). The PSTC is the first public–private partnership created by C-Path to share and validate each others safety testing methods under guidance by the FDA and the EMEA (European Medicines Agency). An excellent review of the reason behind the Critical Path Initiative and discussion of successful consortia has been published recently by Woodcock and Woosley, Director of the Critical Path Initiative, and President of the Critical Path Institute, respectively (Woodcock & Woosley 2008). In view of the Critical Path Initiative, and new findings in bone physiology, this paper aims to review recent publications that together suggest that besides the normal vital functions, the assessment of bone turnover should also play an important role in general safety pharmacology.

Critical Path and Safety Pharmacology

The Critical Path Initiative is firmly linked to normal safety parameters in drug development and thus safety pharmacology. Safety pharmacology has emerged as a distinct discipline during the last decade mainly due to drug failures and enormous progress in biological sciences and technology. Guidelines on safety pharmacology issued by regulatory agencies such as the FDA in the US and the EMEA in Europe are harmonized by the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH), and thus safety pharmacology studies have been defined by both the FDA (http://www.fda.gov/ScienceResearch/SpecialTopics/CriticalPathInitiative/CriticalPathOpportunitiesReports/default.htm) and EMEA (http://www.emea.europa.eu/pdfs/human/ich/053900en.pdf) as follows: Safety pharmacology studies investigate the potential undesirable pharmacodynamic effects of a compound on physiological functions in relation to exposure to the drug in and above the therapeutic range. In particular, the specific objectives in safety pharmacology are, (1) to identify the undesirable pharmacodynamic properties of a substance that might have relevance to its human safety; (2) to evaluate adverse pharmacodynamic and/or pathophysiological effects of a substance that are observed in toxicology and/or clinical studies; and (3) to investigate the mechanism of observed and/or suspected adverse pharmacodynamic effects. In a conventional normal drug development process safety pharmacology includes the assessment of a drug or biological (typically an antibody) on vital functions (termed safety pharmacology core battery by the FDA and EMEA) such as the cardiovascular, central nervous and respiratory systems. As outlined further by the FDA and EMEA, supplemental studies must be conducted outside the safety pharmacology core battery, when there is a cause for concern. This includes effects of the test substance on renal/urinary parameters, on parameters of the autonomic nervous system, the gastrointestinal systems, and other organ systems such as skeletal muscle, immune and endocrine functions. However, effects of the test substance on bone, a major organ, are not included. The exclusion of bone, interestingly, is also reflected by the first consortium created by the Critical Path Initiative, PSTC (see above), which internally has developed safety biomarkers in the following five workgroups: carcinogenicity, kidney, liver, muscle and vascular injury (http://www.c-path.org/pstc.cfm).

Biomarkers

Definition

According to the Biomarker Definitions Working Group, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic interventions (Biomarkers Definitions Working Group 2008). This broad definition includes all diagnostic tests, imaging technologies and all other objective measurements of the status of a biosystem. In drug development biomarkers are used in early efficacy and safety evaluation such as *in vitro* studies (e.g. tissue culture), *in vivo* studies in preclinical settings (animal models), and early-phase clinical trials (patients) to establish 'proof of concept'.

Categories

Different types of biomarkers are needed for different stages of drug development: first, biomarkers that demonstrate the progression of disease and correlate with known clinical indices (e.g. current 'gold standards'); second, biomarkers that capture the effect of an intervention on both known and unknown biological mechanisms associated with clinical outcome and can act as surrogate end points (i.e. changes in these biomarkers can predict clinical outcome); third, diagnostic biomarkers enabling identification of persons with the disease in question; and fourth prognostic and burden-of-disease markers, enabling identification of patients with fast progression and wide disease spread and allowing for selection of those most likely to benefit from treatment. To facilitate research, decrease redundancy and expedite validation of biomarkers in osteoarthritis (OA) research, the 'BIPED' classification scheme for biomarkers, was proposed by Bauer and colleagues, representing the NIH-funded OA Biomarkers Network (Bauer et al. 2006). BIPED stands for burden of disease, investigative, prognostic, efficacy of intervention and diagnostic, and offers

suggestions on optimal study design and analytical methods for use in OA. A schematic outline of the categorization is made in Figure 1, modified from (Karsdal et al. 2009). The BIPED classification thus provides specific biomarker definitions with the goal of improving the ability to develop and analyse osteoarthritis biomarkers, and to communicate results within a common framework. The usefulness of BIPED can be adapted to other fields of disease investigation, and can provide a highly useful framework for discussion of the utility of different biomarkers.

In the following, only the assessment of biological parameters that are referred to as biomarkers will be discussed and it will be limited to metabolites and protein or gene-based biomarkers. Thus many biomarkers such as cholesterol, glucose, triglycerides and HbA1c, have been validated and are easily used in drug development. Others are less well known, e.g. those biomarker tests that assess drug-metabolizing enzymes such as the P450 enzymes or the activity of thiopurine methyltransferase, the assessment of which allows individualized treatments. The latter two biomarkers are examples that illustrate how new tools and the necessary clinical associations develop extremely slowly; it has been known for decades that there are variations in drug-metabolizing enzymes (Finley Austin & Babiss 2006).

Validation

How are biomarkers validated? In the past the 'validity' of preclinical and clinical biomarkers was settled by debate, consensus and the passage of time (Goodsaid & Frueh 2007). Today, with the advent of the Critical Path Initiative, the PSTC, the BIPED classification scheme, and the scheme put forth by Finley Austin and Babiss, the definition of the context for which a biomarker should be qualified can now be more accurately defined. In addition several other initiatives have been instrumental in validation and classification of biomarkers, such as the OMERACT (outcome measure of rheumatoid arthritis in clinical trials) initiative. The OMERACT initiative (OMERATCT 1993) is a description on 'how' and 'what' to measure as clinical outcome or ultimately as clinical endpoint in clinical trials. The 'what' could be any marker that somehow describes the patients: everything from altered gait monitoration to serological biomarkers. The 'how' is how the clinicians measure the marker and whether it follows the current guidelines (Bingham et al. 2009, Conaghan et al. 2009). Validation of a biomarker can refer to the process of retrospectively linking a surrogate endpoint to a clinical endpoint, but more importantly, it can also refer to addressing performance characteristics such as sensitivity, specificity and reproducibility (Biomarkers Definitions Working Group 2008). Thus, the criteria for validation depend on the context and are defined by the nature of the question the biomarker is meant to address (Lesko & Atkinson 2001). A known valid biomarker has been defined as 'a biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is widespread agreement in the medical or scientific community about the physiological, toxicological, pharmacological or clinical significance of the results' (Goodsaid & Frueh 2007). The complexity of validation and evaluation of a biomarker has been reviewed by Lesko and Atkinson (Lesko & Atkinson 2001).

Validated biomarkers of specific physiological mechanisms and efficacy can facilitate rapid progress from phase I to phase II clinical studies on the basis

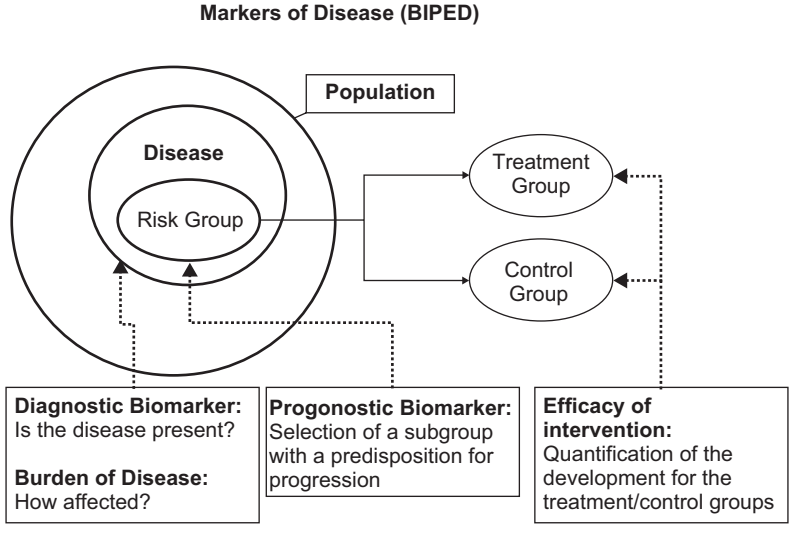

Figure 1. BIPED categorization. Different biomarkers may provide different kinds of useful information. One biomarker may fall in several categories, par example are type I collagen degradation markers often both prognostic and surrogate measure of efficacy markers, thus PE markers.

of the exposure–effect relationship, giving researchers confidence in the maintenance of efficacy in the translation from animal models to humans. Beyond the drug development process, validated biomarkers may also be used in the assessment of the individual patient's response to treatment. By evaluating the results of biomarker assays, clinicians may be able to determine, for example, whether a therapy has had the expected effect, the drug dose or treatment regimen should be changed, disease progression has occurred as predicted, or a new end point has emerged. Currently, only a few validated and generally approved biomarkers are available for use in preclinical studies and clinical trials. For an in depth discussion on validation of biomarkers, the reader is referred to the OMERACT criteria (OMERACT 1993). These guidelines provide an excellent stepwise technical and biological validation classification.

Bone

Introduction to bone and bone markers

Bone turnover is a continuous process that ensures calcium homeostasis and bone quality (Seeman & Delmas 2006). In fact, the total skeleton is averagely completely replaced every 10 years emphasizing the dynamic nature of this organ that in addition may begin to be appreciated with endocrine function.

Bone turnover is mediated by osteoclasts degrading the bone matrix and osteoblasts forming new bone matrix, two processes which, under normal circumstances, are tightly balanced (Karsdal et al. 2007). Thus perturbation of this delicate balance may lead to a pathological condition such as osteoporosis, i.e. pathological bone loss.

In contrast to imaging techniques, biochemical markers of bone turnover obtained in serum or urine samples, show changes in a markedly larger range compared with the imprecision of the assay (<8–10%). Typically for these biochemical biomarkers, a decrease of 50–80% or an increase of 100–200% occurs in days to weeks after initiation of treatment with antiresorptive or anabolic drugs (Hwang et al. 2006, Kung et al. 2006). The respective changes in bone mass range from 6–7% after 2 years of bisphosphonate therapy (Ravn et al. 1999) to 2–3% or less for selective estrogen receptor modulator (SERM) agents and calcitonin (Hansdottir et al. 2004, Overgaard et al. 1989), a fairly small increment relative to the precision error of 1–2% for bone mineral density (BMD) measurements.

In the present context it is important to appreciate the current validity of the biochemical markers of bone turnover, bone formation and bone resorption. Currently there are many important bone markers that each may have a unique value under specific and different contexts. These biochemical markers measuring bone resorption and bone formation, have been developed, applied and validated in preclinical as well as clinical and epidemiological research (Brasso et al. 2006, Coleman et al. 2008, Delmas et al. 2000, Leeming et al. 2006, 2008, Seibel 2005, 2006). A complete listing and discussion of these important biomarkers, each of which may have unique value under specific and different settings, are beyond the scope of the present manuscript, but the authors acknowledge many of these important markers that include but are not limited to; NTX, BS-ALP, PINP, CTX-I, osteocalcin, cathepsin K and TRACP5b. As CTX-I (C-telopeptide of type I collagen) can be easily and non-invasively measured in serum and urine of many species, including humans, it is one of the most widely used biomarkers in preclinical as well as clinical research, and this bone marker will be discussed in more detail below.

The biological rationale for the CTX-I marker is that the osteoclasts mediate bone resorption through active secretion of hydrochloric acid, which is mediated by active proton transport and passive chloride transport into the resorption lacuna (Roodman 1999). In an orchestrated sequence of events, proteases are secreted into the resorption lacunae; cathepsin K is the most important of these proteases (Gelb et al. 1996, Nishi et al. 1999, Sassi et al. 2000). Cathepsin K degrades the organic matrix of the bones, in which type I collagen is the most abundant protein (Seeman & Delmas 2006). The degradation of type I collagen by cathepsin K leads to the release of the CTX-I neoepitope (Garnero et al. 2003, Sassi et al. 2000).

The assessment of bone resorption has proven valid in many different setting in bone biology. As examples, in animal models of bone turnover, such as the FDA-recommended ovariectomized (OVX) rat model for postmenopausal osteoporosis, type I collagen degradation increases with the removal of estrogen, corresponding to increased bone resorption, and can be suppressed by estrogen or SERM treatment (Christgau et al. 2004, Hoegh-Andersen et al. 2004, Schaller et al. 2004). Additional investigations in the OVX model have demonstrated that a reduction in type I collagen degradation in response to antiresorptive treatment leads to improved bone strength (Schaller et al. 2004).

Type I collagen degradation levels increase after menopause (Bonde et al. 1995, Rosenquist et al. 1998) and, importantly, the levels are sensitive to antiresorptive treatment (Leeming et al. 2006, Ravn et al. 2003, Reginster et al. 2001, Rosenquist et al. 1998). This attenuation is rapid (2–3 months or even within hours or days) compared with the very slow increase (18–24 months) of the 'gold standard', i.e. BMD. In patients undergoing antiresorptive treatment, dynamic monitoring of type I collagen degradation during therapy has demonstrated

the efficacy of the intervention through correlation with BMD increase (Christgau et al. 1998, Kim et al. 2005, Okabe et al. 2004). As a result, type I collagen degradation, CTX-I or NTX, are being used in a large number of studies (Black et al. 2006, 2007, Chesnut et al. 2000, Christgau et al. 1998, McClung et al. 2006, Ravn et al. 1999, 2000, 2003, Tanko et al. 2004) as an indicator of decrease in bone resorption and as one component in a fracture-risk prediction. Baseline measurement of type I collagen degradation, which correlates with bone turnover rates (Bonde et al. 1995, Christgau et al. 2000, Reginster et al. 2001), has been shown to have prognostic value as an independent predictor of fracture risk. Combined with BMD age, and/or prior fracture rates, type I collagen degradation measurements can therefore contribute to improvement in the prediction of fracture risk (Garnero et al. 1996).

In summary, type I collagen degradation is a well-validated, sensitive, easily measured and accurate indicator of change in bone turnover and bone resorption. As such, this biochemical marker fulfils many of the BIPED and the Finley/Babiss criteria (see section on Bone as and endocrine organ) and provides proof that it is highly relevant for monitoring the status of bone. An range of possibilities exists for measuring type I collagen degradation, of which CTX-I, NTX-I, or DPD are the most well-established markers (Karsdal et al. 2009, Schaller et al. 2005).

With regard to bone formation, other biochemical bone markers with similar validity are PINP (N-terminal propeptide of type I collagen), bone-specific alkaline phosphatase (BSAP), both early markers of bone formation and osteocalcin, a late marker of bone formation that appears only in the mineralization phase of bone formation (reviewed in Cremers & Garnero 2006, Leeming et al. 2006, Seibel 2005). These bone formation markers provide almost equal information on bone formation.

Summarizing, bone resorption and bone formation can easily be assessed using well-validated biochemical markers. In essence it is the balance between bone resorption and bone formation that provides information on whether bone is lost or gained, and both processes may favourably be assessed.

Bone as an endocrine organ

The exclusion of bone in the FDA's and EMEA's safety pharmacology core battery and the absence of a bone work group in the Critical Path Initiative prompted us to review the following question: is bone turnover controlling other organs? One way to address this question is to look at the *ob/ob* mice, a mutant mouse strain lacking the gene encoding leptin (leptin is a small polypeptide hormone secreted primarily by adipocytes), which has a high bone mass phenotype (Ducy et al. 2000). In 2000 Karsenty and colleagues showed that intracere broventricular infusion of leptin into these *ob/ob* mice normalized bone formation and bone mass (Ducy et al. 2000). Although it has been known for some time that leptin controls body weight through a hypothalamic relay (reviewed in Spiegelman & Flier 1996), the notion that the brain regulates bone was surprising; nevertheless it was confirmed by others (Cornish et al. 2002, Martin et al. 2005), and later reviewed by Karsenty (Karsenty 2006). Subsequently, deletion of the leptin receptor Lepr in neurons of mice formally demonstrated that leptin inhibits bone formation not locally but through neuronal means (Shi et al. 2008). If leptin acts on bone, is there cross-talk between bone and adipocytes, and between bone and other cell types? Some of the answers emerged through elegant studies by Lee et al. who identified osteocalcin as an osteoblast-derived hormone that regulates glucose and energy metabolism via a profound and complex mechanism (Lee et al. 2007). These findings established bone as an endocrine organ, at least in mice, with osteocalcin favouring insulin secretion by β-cells, insulin sensitivity in fat, liver and muscle, and energy expenditure. Interestingly, in contrast to osteocalcin, leptin inhibits insulin secretion. This occurs in part by direct effect on β-cells, but also through indirect mechanisms (Covey et al. 2006, Morioka et al. 2007). A recent study has shown that another indirect inhibition of insulin secretion occurs through inhibition of the bioactivity of osteocalcin by leptin (Hinoi et al. 2008). Other lines of evidence supporting the concept of bone as endocrine organ originate from studies on the $1,25(OH)_2D$ regulating hormone FGF23, a 32-kDa protein, mainly produced and secreted by osteocytes in bone (Liu et al. 2006). FGF23 regulates – in a bone–kidney axis – phosphate, vitamin D and mineral homeostasis (reviewed in Quarles 2008). In addition, the recent finding that poor metabolic control in adolescent type 1 diabetic girls leads to alterations in the growth hormone/insulin-like growth factor (IGF)-1 axis and in IGF-binding protein (IGFBP)-1 levels (the ratio of IGFBP-1/IGF-1 is significantly higher in girls with type 1 diabetes mellitus and predicts smaller bones and reduced cross-sectional areas as compared with age-matched controls), indicates that bone is also an important target organ (Moyer-Mileur et al. 2008). Clearly, emerging functional relationships between adipocytes, neurons and osteoblasts on one side, and kidney cells and osteocytes on the other, suggest the importance of bone turnover in feedback regulation between distant cell types, and illustrate the critical interplay between multiple organs.

This pivotal interplay between many organs has been recognized, yet current biological research is still mainly taking the traditional one-at-a-time approach to study

disease, genes and proteins, the so-called reductionist approach (Arnaud 2006). Many diseases, however, will be treated successfully only with a systemic approach, because diseases such as obesity, diabetes; many heart diseases, hypertension, and osteoporosis are systemic problems. Under these circumstances it is difficult to understand why the FDA or pharmaceutical companies have not yet embraced a more systemic approach, an approach that would include assessing bone on appropriate levels, in detecting adverse effects when developing drugs and conducting clinical trials. Early detection of systemic adverse effects could not only save enormous amount of resources, but also help patients, as illustrated by the development of insulin-sensitivity drugs, thiazolidinediones (TZDs) that turned out to increase fracture risk**,** a fact that was not immediately evident despite early warning signs coming from the use of biochemical bone markers, as outlined in the following. Rezulin (troglitazone), the first compound of the TZD family of oral hypoglycaemic agent to treat type 2 diabetic patients, was launched in the US in spring of 1997, and later that year in Europe (Gale 2001). Within weeks of the European launch, it was withdrawn from the European market based on liver toxicity (Gale 2001), and eventually from the US market in March 2000 (Gale 2006). TZDs are direct agonist ligands for PPARγ (peroxisome proliferator-activated receptor γ) (Lehmann et al. 1995), a receptor that was initially characterized as the master regulator for the development of adipocytes (reviewed in Tontonoz & Spiegelman 2008). Hypothesizing early on that TZDs may affect bone metabolism because adipocytes and osteoblasts originate from common mesenchymal stem cells, Okazaki and colleagues performed a 4-week study in 33 type 2 diabetic patients where they administered troglitazone (Okazaki et al. 1999). Measuring bone formation and resorption markers as well as glycaemic indices, the authors concluded that TZDs first have a direct effect on bone and bone turnover before significantly improving glucose metabolism (Okazaki et al. 1999). Later, evidence from rodent and *in vitro* models suggested that treatment with rosiglitazone, a TZD believed to be less hepatotoxic and thus approved by the FDA hastily in 1999 (Gale 2001), may cause bone loss (Ali et al. 2005, Rzonca et al. 2004, Soroceanu et al. 2004, Sottile et al. 2004). Furthermore, many *in vitro* studies suggest that PPARγ agonists promote adipogenesis at the expense of osteblastogenesis (reviewed in Grey 2008). These warning signs that bone is affected by TZDs, came too late for the ADOPT (A Diabetes Outcome Progression Trial) trial, conducted between April 2000 and June 2002, to include secondary outcome measures in the form of bone parameters, such as BMD or biochemical bone formation and bone resorption markers that would have allowed detection of negative effects on bone (Kahn et al. 2006). Indeed, a postproof note in the main outcome report indicates that further examination of the trial data identified a higher rate of fractures in the group receiving rosiglitazone compared with the groups receiving metformin or glyburide therapy, but men were not affected (Kahn et al. 2006). Rigorous analysis of the ADOPT data came to the conclusion that long-term treatment with rosiglitazone is associated with doubling of the risk of bone fractures in premenopausal and postmenopausal women with type 2 diabetes compared with the two other therapy groups (metformin or glyburide) (Kahn et al. 2008). Several subsequent trials confirmed that TZDs (rosiglitazone and pioglitazone) have negative consequences on bone, at least in women (reviewed in Schwartz 2008). Importantly, when measuring bone turnover markers, significant negative changes in bone are evident very quickly: in a randomized, double-blind rosiglitazone/placebo control trial, osteoblast-specific bone formation markers serum PINP and osteocalcin declined significantly after only 4 weeks, and this effect remained for the duration of the 14-week study in the rosiglitazone group (Grey et al. 2007). There was, however, no change in serum β-CTX (β-C-terminal telopeptide of type I collagen), a marker for bone resorption (Grey et al. 2007). In another rosiglitazone 12-week clinical trial it was shown that bone-specific alkaline phosphatase, an early marker of bone formation, also decreased significantly at the end of the trial (Berberoglu et al. 2007). Both trials indicate that short-term adverse effects by clinically relevant doses of rosiglitazone can be detected early (1–3 months) by bone formation markers, while fracture risk is only noticeable after about 24 months as suggested by the ADOPT results (Kahn et al. 2008). Rosiglitazone has also come under scrutiny due to its association with myocardial ischaemic events (Goldfine 2008, Rosen 2007). Finally, a recent preclinical study used rat-MID osteocalcin and CTX-I in rats to evaluate whether a novel glitazone called balaglitazone suppressed bone formation less than pioglitazone (Henriksen et al. 2009). Interestingly, osteocalcin levels clearly indicated that balaglitazone did not suppress bone formation, while pioglitazone, as expected suppressed osteocalcin levels (Henriksen et al. 2009).

Summarizing, biochemical bone markers predicted adverse effects on bone by TZDs, but these warning signs were ignored in early TZD trials. What exactly then are biochemical markers, and how can they be best used and interpreted?

Other uses of bone turnover markers for safety monitoring

One additional example for the usefulness of biomarkers for safety monitoring is glucocorticoid-induced osteoporosis. Glucocorticoids are widely used for

inflammatory conditions, such as rheumatoid arthritis and inflammatory bowel diseases (Engvall et al. 2008, Vihinen et al. 2008); however their use is associated with severe bone loss due to strongly attenuated bone formation (Caplan & Saag 2009). This attentuation of bone formation leads to a rapid acceleration in the number of fractures in glucocorticoid-treated patients (Caplan & Saag 2009), and patients on glucocorticoids are often treated with antiresorptives (van Brussel et al. 2009). The mode of action of glucocorticoids involves induction of apoptosis in cells belonging to the osteoblastic lineage, i.e. mature bone-forming osteoblasts and osteocytes, and while the effect on bone appears to be dependent on osteoclasts (Kim et al. 2006), there are no indications that the glucocorticoids lead to increased bone resorption (Engvall et al. 2008, Minisola et al. 2008). In summary, apart from during PPARγ-agonist development, biochemical markers of bone turnover have other applications as safety markers. One of these is during use and/or of glucocorticoids, where careful monitoring of bone formation markers, such as osteocalcin or PINP may help avoid some of the detrimental effects in the future (Rosen & Miner 2005), and aid in the identification of new derivative compounds of glucocorticoids that do not have the same negative effect on bone.

Drug safety

The tests that are used to determine drug safety today have not changed for decades, and both the FDA and EMEA have required drug companies to submit the results of two blood tests, i.e. blood urea nitrogen (BUN) and serum creatinine to evaluate renal toxicity. Although drug companies have developed newer safety testing methods, these methods have not generally been accepted by the FDA or the EMEA. However, the PSTC formed by the Critical Path Initiative succeeded in establishing seven key proteins found in urine as new biomarkers to indicate possible kidney injury (http://www.fda.gov/bbs/topics/NEWS/2008/NEW01850.html). The new biomarkers, accepted by the FDA and EMEA in May 2008 are kidney dysfunction markers KIM-1, albumin, total protein, B_2-microglobulin, cystatin C, and tumour and cancer progression markers clusterin and trefoil factor-3, respectively. Again, none of the newly accepted biomarkers include biochemical markers that address bone status despite mounting evidence (see section on Biomarkers, Categories) that bone is a systemic organ that may react very quickly to adverse effects in clinical trials.

The FDA and EMEA, even the World Health Organization (WHO), have not yet adopted the usefulness of any biochemical marker that assesses bone turnover or bone status despite the fact that they are presently the most advanced with respect to matrix remodelling (Rousseau & Delmas 2007, Schaller et al. 2005). This is somewhat surprising, particularly in view of proclaiming the current decade the Bone and Joint Decade, an initiative that was launched by the United Nations, WHO and 37 countries to improve the lives of people with musculoskeletal disorders (http://www.boneandjointdecade.org). The reason for rejecting the acceptance of the usefulness of biochemical bone markers by these agencies and, in fact, many professional societies is three-fold (Meier et al. 2009): first, many biochemical bone markers exhibit within-subject variability; second, many countries lack pivotal quality control programmes for bone turnover markers, and this deficiency is aggravated by poor standardization of many assays including those by commercial laboratories; and third, a lack of widely accepted reference ranges that may leave clinicians with questions of how to interpret given results. However, safety pharmacology does not look at individual patients, but at large populations, and therefore within-subject variability is statistically eliminated. This is the main reason why so many clinical trials that tested the safety and efficacy of osteoporosis treatments successfully used CTX-I bone markers as outlined above. Furthermore, several other bone remodelling markers have been shown to be useful; a non-exhaustive list includes NTX-I (Garnero et al. 2000b, Gertz et al. 1998), Dpd (Robins et al. 1994), BAP (Broyles et al. 1998), and osteocalcin (Rosenquist et al. 1995, Tanko et al. 2004). Quality assurance and standardization can be achieved by a quality assessment scheme similar to what DEQAS (Vitamin D external quality assessment scheme) has done to ensure the analytical reliability of 25 hydroxyvitamin D (25OHD) and 1,25 dihydroxyvitamin D (1,25(OH2D) assays. DEQAS has been monitoring the performance of 25OHD assays since 1989, has >200 registered laboratories worldwide (Binkley et al. 2008), and thus can provide international perspective on methodology and clinical interpretation (Carter et al. 2004a, b). Guidelines and recommendations about how to use biochemical bone markers have been published many times since 2000, and this should facilitate consensus (Bergmann et al. 2009, Coleman et al. 2008, Delmas et al. 2000, Garnero et al. 2000a, Roux et al. 2005, Seibel 2006).

Conclusion

In conclusion, there are many compelling reasons why safety pharmacology should embrace biochemical bone markers, among which CTX-I, PINP, BSAP and osteocalcin are probably the most suitable. Thus an open question for the FDA and EMEA to consider may be 'Should biochemical markers of bone turnover be considered standard practice for safety pharmacology?'

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Declaration of interest

All authors declare that the affiliation indicates full disclosure. Morten A. Karsdal owns stocks in Nordic Bioscience.

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