

Review Article

Cellular and molecular effects of thiazolidinediones on bone cells: a review

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Abstract: Thiazolidinediones represent a class of molecules used in the treatment of type 2 diabetes mellitus. Despite interesting effects in lowering blood glucose and HbA1c levels durably, an augmentation of the fracture risk in women has emerged in the past years. This review is providing the readers with information about the cellular and molecular mechanisms involved in bone and bone cells in response to these drugs.

Keywords: Thiazolidinediones, osteoclast, osteoblast, osteocyte, PPAR γ , diabetes mellitus

Introduction

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin (type 1 diabetes mellitus) or when the body cannot effectively use the insulin it produces (type 2 diabetes mellitus). Worldwide it is estimated that 220 million people suffer of diabetes mellitus and among all the diabetic patients, almost 90% suffer of type 2 diabetes. Type 2 diabetes mellitus (T2DM) is largely the results of excess body weight and physical inactivity and is characterized by insulin and glucose intolerance associated with hyperglycemia and hyperinsulinemia leading to serious damage of the heart, blood vessels, eyes, kidneys, and nerves. First line therapy include life style intervention and when this action is not enough to lower sufficiently blood glucose levels, additional therapies including insulin, insulin secretagogues (sulphonylurea, incretins) or blood glucose lowering molecules (biguanides, α -glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase inhibitors, glinides and amylin agonists) are required [1]. Among all these molecules, only thiazolidinediones (TZDs) have insulin-sensitizing properties and are the most efficient in controlling durably T2DM [2, 3]. However, there are accumulating evidences that treatment with thiazolidinediones cause bone loss and increase fracture risk in humans, spe-

cifically in women although several studies reported the same effects in men [4, 5].

Molecules of the thiazolidinedione family

Several molecules, marketed and non-marketed, belong to the thiazolidinedione family (**Figure 1**). All members have in common a 5-benzyl-1, 3-thiazolidine-2, 4-dione group and differ by their side chain. Among all thiazolidinedione members, pioglitazone is currently the only molecule marketed in Europe but there is a debate concerning its possibility to increase the frequency of bladder cancer [6]. Rosiglitazone was withdrawn from the european market in October 2010 due to cardio-vascular adverse events and troglitazone was withdrawn worldwide in 2000 due to severe liver problems. The vast majority of thiazolidinedione members have never been marketed but they have all in common to bind and activate partially or fully the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR γ).

PPAR γ and its ligands

PPAR γ is a member of the PPAR subfamily of nuclear receptor. In humans, the *ppar γ* gene is located on chromosome 3 at the 3p25 locus. Differential promoter usage and alternative splicing produce four variants, including two

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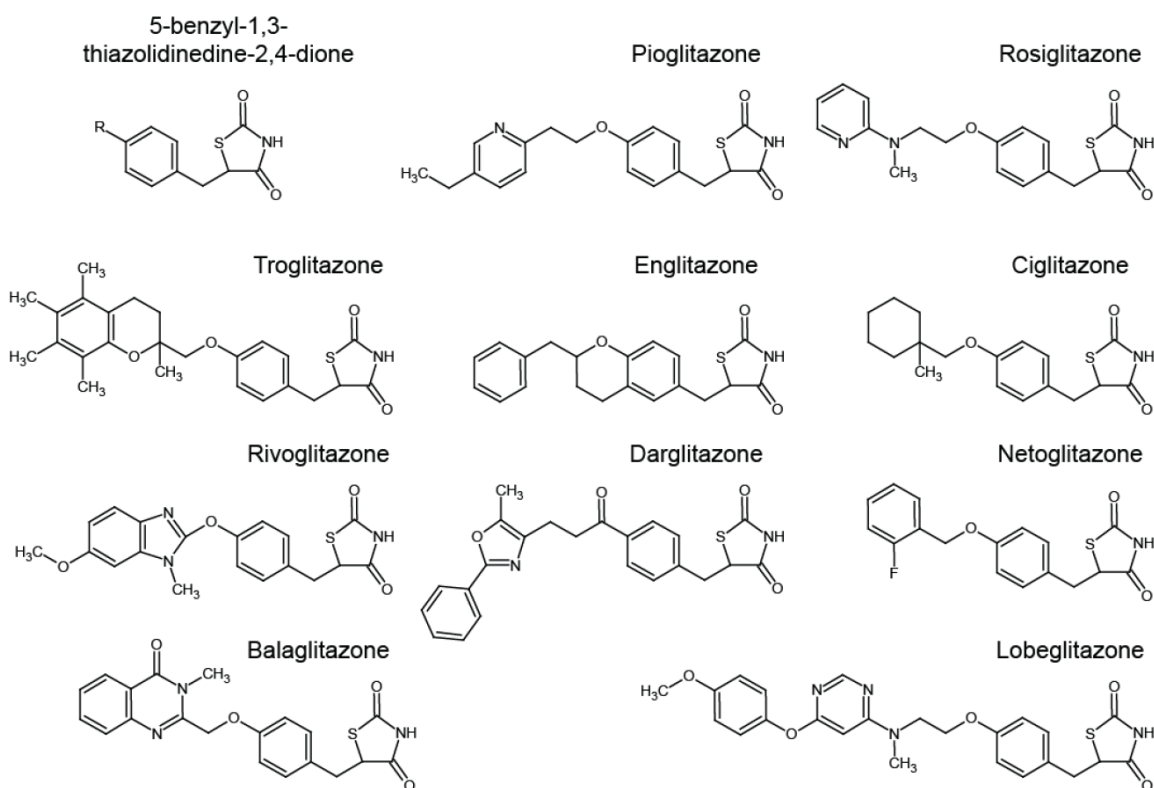


Figure 1. Members of the TZDs family.

major forms of the protein, PPAR γ 1 and PPAR γ 2. PPAR γ 1 is expressed in a large range of tissues, including the liver, skeletal muscle, adipose tissue and hematopoietic cells. The expression of PPAR γ 2, that contains 30 additional amino acids in its N-terminus extremity, is restricted primarily to adipocytes, stromal cells and osteoblasts [7]. Upon activation, PPAR γ heterodimerize with a member of the retinoid X receptor (RXR) family and the heterodimer PPAR γ /RXR activates the transcription of target genes [8]. PPAR γ acts primarily as a master gene in metabolic regulation through stimulating insulin sensitivity, glucose-lowering and lipid uptake and storage in peripheral organs such as skeletal muscle, liver and adipose tissue. In particular, PPAR γ is the key regulator of adipogenesis and adipocyte metabolism and also exerts anti-inflammatory and anti-atherosclerotic actions. PPAR γ activity can be modulated directly or indirectly at different levels of the activation cascade. This topic has been extensively studied and is reviewed in Lucini et al [9].

Fatty acids and eicosanoids derivatives bind and activate PPAR- γ at micromolar concentrations and represent natural ligands for this receptor. Clearly, PPAR- γ prefers polyunsaturated fatty acids including linoleic acid, linolenic acid, arachidonic acid and eicosapentaenoic acid [10]. The micromolar affinity of these metabolites is in range with their serum concentrations. However, their intracellular concentration ranges are unknown. Conversion of linoleic acid to 9-HODE and 13-HODE by 15-lipoxygenase can provide additional micromolar PPAR- γ agonists [11]. A prostaglandin D₂-derivative, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂), was demonstrated to be a relatively weak (2-5 μ M) PPAR- γ ligand and agonist [12, 13], although the physiological relevance of this ligand is unclear because cellular concentrations cannot be accurately determined. An oxidized phospholipid, hexadecyl azelaoyl phosphatidylcholine, was shown to bind PPAR- γ at nanomolar concentrations [14].

The first association of thiazolidinediones with PPAR γ was reported by Lehmann et al. in 1995

Table 1. Ligand binding affinities for PPAR- γ [47-49]

	EC ₅₀ (μ M)
Rosiglitazone	0.2
Pioglitazone	0.69
Troglitazone	0.78
Ciglitazone	3
Englitazone	13
Netoglitazone	8
Balaglitazone	1.35
Lobeglitazone	0.22

[15]. These authors highlighted that TZDs bind and activate PPAR γ in a dose-dependent manner. Among all developed TZDs, rosiglitazone has the highest binding affinity at a nanomolar concentration range (Table 1).

Bone loss and increased skeletal fragility in TZD-treated patients

Thiazolidinediones are used in patients with diabetes mellitus and as such it is a prerequisite to appreciate the effects of diabetes mellitus on bone to understand how thiazolidinediones may favor a skeletal fragility. It is well admitted that type 1 diabetes induce skeletal fragility and osteopenia. Far less is known for type 2 diabetes. Although the bone mineral density is similar or higher than non-diabetic volunteers, it appears that T2DM patients present an increased skeletal fragility. This topic has already been reviewed by Hofbauer et al. [16] and will not be detailed herein.

TZDs have been the centre of investigation during the past years and several randomized clinical trials have been undertaken comparing TZDs with placebo. All started with the results of the ADOPT study that investigated the effects of rosiglitazone over metformin or glyburide on metabolic parameters. Results from the ADOPT study highlighted the existence of an increased risk of developing bone fractures in women, but not men, treated with rosiglitazone compared with women treated in the other arms of this study [5]. Similar results with pioglitazone were also released in a note by Eli Lilly Canada Inc [17]. Schwartz et al. examined the association between TZD use (pioglitazone, rosiglitazone and troglitazone) and bone loss of elderly Americans [18]. Sixty-nine patients reported TZD use

during a 4-year period of observation. Bone loss was accelerated, by 0.6-1.2% per year, at the trochanter, whole body, and lumbar spine in diabetic women who reported any TZD use compared with those who did not. Longer duration of TZDs and higher compliance with therapy were associated with more rapid rates of bone loss. However, none of the 32 male included in this trial who reported TZD use presented difference in bone mass. Grey et al. reported the results of a 14-week randomized clinical trial comparing rosiglitazone (8 mg/day) with placebo in 50 post-menopausal women who did not have diabetes mellitus or osteoporosis at the beginning of the study [19]. In the rosiglitazone group, each of two specific markers of bone formation (osteocalcin and procollagen type 1 N-telopeptide) declined by 10-12% compared with the placebo group while there was no change in serum β -CTX, the marker of bone resorption. These changes in bone turnover were accompanied by a significant 2% decline in total BMD in the rosiglitazone-treated group, even with the short time frame of this trial. Another study enrolled 56 postmenopausal women with T2DM who were randomized to rosiglitazone (4 mg/day) or dietary advice for 12 weeks [20]. Bone specific alkaline phosphatase declined by 21% while urinary deoxypyridinoline did not change. These results demonstrate the alterations of bone metabolism (decreased bone formation whilst bone resorption is unchanged) associated with the use of clinically relevant dose of rosiglitazone in a relatively short time. These results or somehow similar to those induced by treatment with glucocorticoids [21].

Thiazolidinediones and bone cells

Three different categories of cells participate to the physiological bone remodeling, osteoclasts, the bone-resorbing cells; osteoblasts, the bone-forming cells and osteocytes, cells embedded in the bone tissue that control bone resorption and bone formation.

Osteoclasts

Osteoclasts, the multinucleated giant cells that resorb bone, develop from hematopoietic cells of the monocyte-macrophage lineage. Effects of PPAR γ ligands and especially TZDs have been extensively studied on this specialized bone cells. Okazaki et al reported that in a model of mouse bone marrow culture, TZDs significantly

increased the expression of PPAR γ 2, but not PPAR γ 1 in bone marrow cells [22]. Parathyroid hormone (PTH) and 1,25(OH) $_2$ D $_3$ are well-known inducers of osteoclast formation in bone marrow culture [23]. When conjointly, bone marrow cells were treated with an inducer of osteoclast formation and a TZD, a significant inhibition of osteoclast formation was noticed and troglitazone was reported to significantly inhibit bone resorption in this model [22]. Further studies by other groups confirmed that treatments of osteoclast precursors (human or murine) with PPAR γ agonists resulted in decreased osteoclast formation and resorption [24, 25]. Recently, Wan et al reported an unexpected effect of rosiglitazone on osteoclasts [26]. Mice with a deletion of PPAR γ in osteoclasts exhibited an increase in osteoclast differentiation and bone resorption. Mechanistically, in this model PPAR γ activation by rosiglitazone directly potentiates RANKL induction of c-fos, an essential mediator of osteoclastogenesis and requires PGC1beta [26, 27]. Increased bone resorption was also observed in mice treated for 8 weeks with darglitazone [28].

Osteoblasts

Osteoblasts derive from mesenchymal stem cells (MSC). Under different stimuli, MSCs can give rise to osteoblasts, chondrocytes, muscle cells, fibroblasts and adipocytes depending on which transcription factor is activated [29]. As such activation of cbfa-1/runx-2 leads to the differentiation of MSC into osteoblasts whilst the activation of PPAR γ 2 leads to the increased expression of adipocyte-specific genes and differentiation of MSC into adipocytes [30]. The differentiation of murine embryonic stem cells into osteoblasts may be attenuated by interference with PPAR γ signal. In fact the use of synthetic siRNA targeting PPAR γ suppressed adipocyte differentiation and successfully induced osteoblastic differentiation, even in the absence of osteoblastogenic factors [31]. On the other hand, activation of PPAR γ 2 leads to a sequence of events toward adipogenesis and a suppression of osteoblastogenesis [32]. As TZDs are PPAR γ agonists, it has been postulated that TZDs would promote adipogenesis at the expense of osteoblastogenesis. Lecka-Czernik et al, reported that as compared with vehicle, mesenchymal progenitor cells treated in vitro with 1 μ M rosiglitazone exhibited a 27% increase in adipogenesis and a 47% decrease in osteoblas-

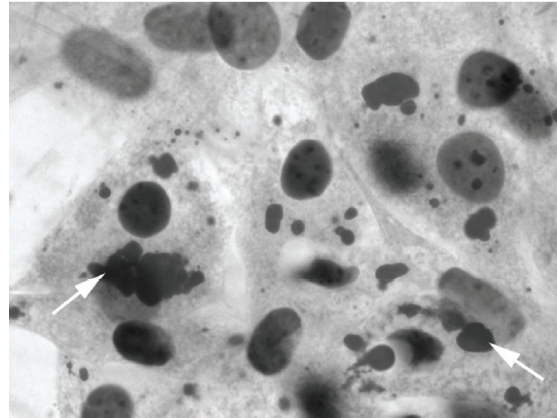


Figure 2. C3H10T1/2 cells cultured with pioglitazone. Some cells have accumulated intracytoplasmic vacuoles filled with lipids (arrows) and their nucleus is smaller and darker than cells without lipids. Oil red O staining counterstained with haematoxylin.

togenesis [33]. Further studies demonstrated that this mechanism occurs also in vivo and Rzonca et al reported an increase in the fat volume and a decrease in the bone volume in the bone marrow of rosiglitazone-treated mice [34], a phenotype similar to what has been published in human clinical trials. We also found that treatment of mesenchymal cells with TZDs resulted in increased adipogenicity in the culture (**Figure 2**). Image analysis of these cultures revealed severe modification of the nucleus in TZD-treated cells with reduction in the nucleus area and increased condensation of the heterochromatin resulting possibly in reduced transcriptional activity in these cells [35].

Osteocytes

Osteocytes are the most abundant cells of the bone and it is estimated that the total number of osteocytes in adult human bone is approximately 10 times higher than osteoblasts [36]. Osteocytes are cells embedded in the bone matrix. Originally, osteocytes arise from the differentiation of a subset of osteoblasts that work slower and are embedded during the deposition of a new bone matrix. However, our current knowledge of the effects of TZDs on osteocytes lags behind what we know of both osteoblasts and osteoclasts. Nevertheless, Soroceanu et al investigated this matter in vivo in the presence of rosiglitazone. These authors observed an increase in apoptosis of the osteoblast/osteocyte cells after treatment with TZDs but

unfortunately did not separate osteoblasts from osteocytes in their quantification [37]. Recently, we have shown that pioglitazone, rosiglitazone and troglitazone induce significantly osteocyte apoptosis in a dose-dependent manner [38]. Moreover, TZD-treated osteocytes exhibit higher levels of sclerostin, an inhibitor of the Wnt signaling pathway. Activation of the Wnt signaling pathway is a crucial event in the synthesis of a new bone matrix and as such it is possible that through the release of this soluble mediator, TZD-treated osteocytes could decrease bone formation and exacerbate low bone mass.

More than 77% of the women enrolled in the ADOPT study were post-menopausal [5] and recent evidences highlighted that a crosstalk between estrogen receptor (ER) and PPAR γ exist. For example ER can bind to PPAR γ responsive element in the promoter of targeted genes and this way negatively interferes with PPAR γ -mediated transcription [39]. ER also shares several co-activator molecules with PPAR γ and as such recruitment of these mediators by the ER complex limits their availability to associate with PPAR γ [40]. We studied the effects of estrogens in TZD-treated osteocytes in vitro [38]. Estrogen-treated osteocytes were protected from TZD-induced apoptosis and these cells stopped totally to release sclerostin in their environment. Overall, it seems that the lack of estrogen potentiates the negative effects of TZDs on osteocytes and these results suggest that TZD therapy should not be envisaged in post-menopausal women. On the other hand, far less is known about the capacity of androgens to protect osteocytes from the negative effects of TZDs. Questions concerning the use of TZDs in androgen-deprived males remain and studies are urgently needed to fully understand the bone safety of these drugs in these patients.

Indirect effects

Although it appears clear that TZD could directly affect the metabolism of bone cells, it is also tenable that TZDs exert skeletal actions through indirect means. TZDs activate PPAR γ in adipose tissue and contribute to modify the pattern of expression of adipokines, such as leptin and adiponectin [41, 42]. These two molecules are known to act on the bone homeostasis [43]. TZDs also contribute to decrease the circulating levels of insulin and IGF-I, two anabolic agents for osteoblasts [44, 45]. Furthermore, pancre-

atic stimulation of PPAR γ results in reduced release of amylin, a peptide known to restrain osteoclastogenesis [46].

Conclusion

In conclusion, TZDs participate to increase adipogenesis at the expense of osteoblastogenesis and as such probably contribute to the reduction of bone mass observed with TZD treatment. TZDs also contribute to the death of osteocytes and the increased expression of sclerostin that in return certainly could emphasize and aggravate the reduction in bone formation. On the other hand, effects of these drugs on osteoclasts seem more controversial and surely require more studies before totally elucidating in which proportion these cells participate to the skeletal phenotype observed with TZD treatment.

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