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REVIEW ARTICLE

Do thiazolidinediones still have a role in treatment of type 2 diabetes mellitus?

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Thiazolidinediones have been introduced in the treatment of type 2 diabetes mellitus (T2DM) since the late 1990s. Although troglitazone was withdrawn from the market a few years later due to liver toxicity, both rosiglitazone and pioglitazone gained widespread use for T2DM treatment. In 2010, however, due to increased risk of cardiovascular events associated with its use, the European Medicines Agency recommended suspension of rosiglitazone use and the Food and Drug Administration severely restricted its use. Thus pioglitazone is the only thiazolidinedione still significantly employed for treating T2DM and it is the only molecule of this class still listed in the American Diabetes Association-European Association for the Study of Diabetes 2012 Position Statement. However, as for the other thiazolidinediones, use of pioglitazone is itself limited by several side effects, some of them potentially dangerous. This, together with the development of novel therapeutic strategies approved in the last couple of years, has made it questionable whether or not thiazolidinediones (namely pioglitazone) should still be used in the treatment of T2DM. This article will attempt to formulate an answer to this question by critically reviewing the available data on the numerous advantages and the potentially worrying shortcomings of pioglitazone treatment in T2DM.

Keywords: PPAR-gamma agonist, thiazolidinediones, type 2 diabetes

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Introduction

Thiazolidinediones are selective ligands of the nuclear transcription factor peroxisome-proliferator-activated receptor γ (PPARγ). These are a subfamily of the 48-member nuclear-receptor superfamily which, once activated by ligand binding, bind to DNA in complex with the retinoid X receptor (RXR), thus modulating the transcription of a number of specific genes [1]. Since the late 1990s, thiazolidinediones have been introduced in the treatment of type 2 diabetes mellitus (T2DM), mostly as second-line agents, in combination with metformin. Three thiazolidinedione molecules have been used in clinical practice over the last 15 years: troglitazone, rosiglitazone and pioglitazone. Troglitazone, the first thiazolidinedione approved for clinical use in 1997, was withdrawn from the market 3 years later, after reports of a few individual cases of liver injury and failure associated with use of the drug [2]. On the other hand both rosiglitazone and pioglitazone gained widespread employment for T2DM treatment in the early years of the last decade. Then, mostly prompted by the results of a meta-analysis showing association of its use with increased cardiovascular events [3], rosiglitazone underwent very close scrutiny by both Food and Drug Administration (FDA) and European Medicines Agency (EMA) [4].[a] Additional data were gathered and, as a result of further publications suggesting increased cardiovascular risk with the use of rosiglitazone [5], in September 2010 the FDA put use of it under very strict restriction while the EMA recommended suspension of the drug [6]. Pioglitazone, therefore, is the only thiazolidinedione still significantly adopted and is the only molecule of this class still listed in the American Diabetes Association (ADA)-European Association for the Study of Diabetes (EASD) 2012 Position Statement on T2DM treatment [7]. However, as for the other thiazolidinediones, pioglitazone too is limited by several side effects, some of them potentially dangerous [8]. This, together with the development of novel therapeutic strategies approved in the last couple of years, such as the glucagon-like-peptide-1 (GLP-1) receptor agonists or the dipeptidyl peptidase-IV (DPP-IV) inhibitors, has made it debatable whether or not thiazolidinediones (now meaning pioglitazone) should still be used in the treatment of T2DM. The question is not an easy one to answer in the light of the various unique advantages of thiazolidinedione treatment, such as durability and potential disease progression modifying mechanisms of action [9]. This article, based on a PubMed literature search including the terms 'thiazolidinediones', 'pioglitazone', 'rosiglitazone' and 'diabetes treatment', will attempt to formulate an answer to this question by critically reviewing the available data as to the

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[a]European Medicines Agency Avandia: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2010/09/WC500096996.pdf.

numerous advantages and potentially worrying shortcomings of pioglitazone treatment in T2DM.

Thiazoldinediones: The Advantages

Effects on Glucose Metabolism

Pioglitazone was approved as a diabetes drug because of its ability to improve blood glucose levels and HbA1c in subjects with T2DM [10,11]. Pioglitazone efficacy in improving metabolic control in diabetes is at least not inferior to that of sulfonylureas, glinides or DPP-IV inhibitors [10,12]. In at least two trials pioglitazone appeared actually superior to gliclazide [13,14] in improving glucose metabolism in diabetic patients. Similarly, pioglitazone proved significantly superior to the DPP-IV inhibitor sitagliptin in reducing HbA1c levels in drug-naïve people with type 2 diabetes [15] as reported in the ADA/EASD consensus expert statement [7]. Furthermore, in drug-naïve people with type 2 diabetes , at a 2-year follow-up, pioglitazone treated subjects maintained acceptable metabolic control (HbA1c < 8%; 64 mmol/mol) in a significantly larger proportion than did gliclazide-treated subjects [14,16]. Similar results were achieved when the two drugs were used in addition to metformin [17].

The durability of the antihyperglycaemic effects of rosiglitazone was investigated in drug-naïve patients. The A Diabetes Outcome Progression Trial (ADOPT) found that in the long term (4 years) rosiglitazone-treated patients experienced significantly greater durability in terms of maintenance of acceptable fasting plasma glucose levels, although, if considered as absolute values, the differences in the glycaemic control achievable with rosiglitazone or metformin were small [18]. Thus, although it might be slower, response to thiazolidinedione treatment is not inferior to other treatment strategies and, most important, it is long-lasting and achieved at a very low risk of hypoglycaemia [19].

In addition to provide sustained glycaemic control in people with type 2 diabetes, thiazolidinedione treatment seems able to slow the progression towards T2DM in individuals with impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG).

Both troglitazone and rosiglitazone have been shown to reduce IGT conversion to T2DM [20,21]. As for pioglitazone, in women of Latin American descent with a history of gestational diabetes, it significantly reduced the incidence of T2DM later in life [22]. Furthermore, in the ACT NOW study [23], 602 IGT subjects were randomized to receive either pioglitazone or placebo: during the 2.4 years median follow-up period, the annual incidence rate for T2DM was 2.1% in the pioglitazone group and 7.6% in the placebo group. This represents a stunning 72% decline in the risk of developing diabetes in the population studied. It is currently thought that T2DM is a relentlessly progressive disease [24]. Therefore a drug inducing sustained acceptable glucose control and associated with a significant decrease in conversion from IGT to overt diabetes, could be viewed as a disease progression modifying agent rather than as a simple glucose-lowering agent. To affect T2DM progression, a drug must be able to decrease insulin resistance and, more important, slow down β-cell failure [25]. As discussed above, thiazolidinediones have showed acceptable durability and seem able to drastically reduce conversion from IGT to T2DM. Considerable evidence, from *in vitro*, animal and small human studies supports the notion that thiazolidinediones can alleviate insulin resistance and might possibly exert both direct and indirect protective actions on the β-cell [26–28].

In the adipose tissue, thiazolidinedione-induced PPARγ activation increases the capacity for free fatty acid (FFA) storage, thus reducing FFA concentration in plasma and excessive accumulation of FFA in the liver [29,30]. Decreased FFA accumulation enhances insulin action in the liver and in the skeletal muscle [31]. By activating PPARγ in the adipose tissue, thiazolidinediones decrease inflammatory cytokines, such as tumour necrosis factor-α (TNF- α) and interkeukin-6 (IL-6), while increasing circulating levels of adiponectin [32,33]. Since inflammatory cytokines hinder insulin action [34], this also results in improved insulin sensitivity. As suggested by several *in vitro* studies, a decrease in FFA levels and in circulating inflammatory cytokines should not only alleviate insulin resistance, but also improve β-cell function [35,36]. Besides, thiazolidinediones might have direct protective effects on β-cells. β-Cells do express PPARγ [37] and PPARγ agonist exposure prevents amyloid-induced apoptosis in cultured β-cells [27]. Furthermore, in different animal models of diabetes, thiazolidinedione treatment preserved the pancreatic islets structure and β-cell function [26,38]. Finally, *in vivo*, in people with type 2 diabetes, Gastaldelli et al. [28] reported that pioglitazone treatment significantly improved the disposition index, a proxy of β-cell function. In summary, pioglitazone might be able to act on both key elements of T2DM pathophysiology, namely progressive β-cell failure and insulin resistance.

Effects on Cardiovascular Risk

Several pre-clinical observations would support the notion that thiazolidinediones have a protective effect against atherosclerosis. PPARγ exert crucial functions in modulating vascular inflammation [39] and, in animal models, their activation by thiazolidinediones reduces expression of inflammatory markers such as TNF-α or metalloproteinase-9 in the arterial wall [40]. Furthermore, thiazolidinediones inhibit TNF-α induced VCAM-1 expression in cultured endothelial cells and decrease the homing of monocytes in ApoE-deficient mice [41]. Thiazolidinediones also inhibit vascular smooth muscle cell proliferation and, in these cells, downregulate expression of angiotensin II type 1 receptor [42,43]. Furthermore, it has been very recently reported that rate of carotid intima-media thickness (CIMT) progression in individuals with IGT was reduced by nearly half during pioglitazone treatment, independently of known atherosclerosis risk factors improvement. This suggests a possible direct vascular benefit of pioglitazone [44].

More controversy attaches to the role of PPARγ ligands in modulating macrophage low density lipoprotein (LDL) receptors. Thiazolidinediones have been reported to increase expression of the macrophage LDL receptor CD36 [40]. This should lead to increased macrophage LDL content in response to pioglitazone treatment. On the contrary, however, PPARγ activation has been reported to reduce macrophage glycated lipoprotein uptake and LDL accumulation. Thus, it

might be, as proposed by Zhang and Chawla [45], that PPARγ activation reduces accumulation of atherogenic oxidized LDL in the vessel wall by increasing both macrophage uptake and efflux via upregulation of CD36.

In the intact animal, thiazolidinedione treatment displayed protective effects against experimentally induced myocardial or brain ischemia [46–48]. Moreover, albeit with important differences among the different molecules as we will discuss later, thiazolidinediones positively affect traditional cardiovascular risk factors such as lipid profile and blood pressure [48]. Thiazolidinediones also appear to positively affect a number of non-traditional markers associated with cardiovascular outcomes. As reviewed by Marx and Walcher [49], thiazolidinedione treatment in humans is associated with decreased plasma C-reactive protein and PAI-1 levels, increased adiponectin concentrations and improved endothelial function and albuminuria.

Thus, considering the wealth of data providing a rationale for the possible positive effect of thiazolidinediones on cardiovascular risk, one can understand the shock of the scientific community upon the publication of the first meta-analysis reporting increased cardiovascular risk associated with the use of rosiglitazone [3]. No large, definitive cardiovascular outcomes trials were conducted with rosiglitazone and this ignited the debate even more, leading to publication of several meta-analyses looking at rosiglitazone use and cardiovascular risk [50]. Out of 13 such meta-analyses, 8 reported a significant increase in the risk of myocardial infarction (MI) or cardiovascular death with the use of rosiglitazone [50]. An update of the original meta-analysis by Nissen and Wolski published by the same authors 2 years later [51] confirmed a statistically significant 28% increase in the risk of MI associated with rosiglitazone, although it failed to show a parallel increase in cardiovascular death.

Unlike the cumulative evidence suggesting adverse effects by rosiglitazone on cardiovascular outcomes, no negative trend seems to exist for pioglitazone, which appears by contrast to reduce cardiovascular risk. A meta-analysis on pioglitazone and cardiovascular safety actually showed a significant 18% reduction in the risk of a composite of cardiovascular outcomes and a non-significant trend towards a reduction in the risk of MI [52]. Subsequently published additional meta-analyses confirmed a picture of cardiovascular protection associated with pioglitazone [53,54].

Furthermore, in a 74-week average follow-up study, pioglitazone, as compared to glimepiride, prevented the progression of carotid thickening [55]. Similarly, in the PERISCOPE study, 18 months of pioglitazone treatment, as compared to glimepiride, significantly decreased coronary atherosclerosis as evaluated by Intra-Vascular coronary Ultra-Sound [56]. Evidence supporting a pioglitazone-associated cardiovascular protective effect also derives from a randomized, placebo-controlled intervention trial. In the PROactive study, over 5000 patients with T2DM and previous cardiovascular events were randomized so as to assume pioglitazone or placebo on top of their current treatment. Although the primary endpoint [a composite of death, non-fatal MI, acute coronary syndrome, stroke, major leg amputation, coronary or leg revascularization] showed only a non-significant 10% reduction in the pioglitazone arm, a significant 16% reduction in the secondary endpoint (a composite of death, non-fatal MI and stroke) was associated with pioglitazone treatment in the 4-year follow-up period [57]. Furthermore, in patients with previous MI, pioglitazone significantly reduced the risk of another MI by 28% [58]. In patients with a previous stroke, pioglitazone decreased by almost 50% the chances of a second stroke [59]. In agreement with the PROactive results, a retrospective study on a large cohort of T2DM patients from a UK general practice research database, found treatment with pioglitazone, but not rosiglitazone, to be associated with a significantly reduced risk of all-cause mortality when compared to metformin [60]. Finally, in a recently published observational study on a large cohort of patients initiated on second-line glucose-lowering agents after metformin failure, a metformin plus pioglitazone combination resulted in significantly lower adjusted Hazard Ratios for all-cause mortality as well as for a combined endpoint composed of all-cause deaths, MI, stroke and cancer [61].

On the basis of the available evidence, it does appear therefore that a profound difference exists between rosiglitazone and pioglitazone as pertains to their cardiovascular effects.

And indeed, a meta-analysis of 16 observational studies directly comparing the risk of cardiovascular outcomes for rosiglitazone and pioglitazone among patients with T2DM showed that, compared to pioglitazone, use of rosiglitazone was associated with a statistically significant increase in the odds of MI, congestive heart failure and death [5]. Thus, although without the ultimate proof of randomized, head to head trials, it appears that while rosiglitazone might be cardiotoxic, pioglitazone is very likely cardioprotective. As to the reasons for this difference, the different impact of the two molecules on the lipid profile has been implicated.

Both pioglitazone and rosiglitazone seem to increase LDL cholesterol: this increase, however, appears to be significantly lower with pioglitazone; furthermore, triglyceride levels tend to increase with rosiglitazone while pioglitazone treatment decreases triglycerides and favourably changes LDL particle size and concentration in plasma [62]. Finally, HDL cholesterol, which is strongly correlated with cardiovascular outcome in the PROACTIVE study [63], increases more with pioglitazone than with rosiglitazone [62,64]. In the CHICAGO study, the beneficial effect of pioglitazone on HDL was an independent predictor of CIMT progression reduction [65].

The mechanisms underlying the differential effects of pioglitazone and rosiglitazone on serum lipids might be rooted in pioglitazone's ability to partially activate PPAR-α, a feature which rosiglitazone does not share [66–68]. PPAR-α, by regulating the expression of proteins involved in FFA transport and β-oxidation, plays a pivotal role in the regulation of lipid and glucose metabolism [69]. In a study by Szapary et al., pioglitazone treatment for 12 weeks significantly increased Apo-AII [70], while Qin et al. showed that pioglitazone stimulates Apo-AI production in HepG2 cells through PPAR-α activation [66]. The same authors have shown that pioglitazone increases apoA-II synthesis and mRNA expression in HepG2 cells. These findings support the notion that pioglitazone increases apoA-I and apoA-II through its PPAR-α binding.

Above all, however, it must be kept in mind that pioglitazone and rosiglitazone affect gene expression in a profoundly

different fashion. It has been shown that rosiglitazone is able to activate 65 genes: of these 25 are not in common with the 52 activated by pioglitazone. Rosiglitazone also represses 140 genes: of these 83 are not in common with the 70 repressed by pioglitazone [71]. We still have a lot to learn about which processes are actually regulated by these genes, but the striking difference in the activation/repression pattern between the two molecules might very well be the underlying mechanism behind their different impact on cardiovascular risk.

Effects on Restenosis After PTCA

Thiazolidinediones might have a protective effect on restenosis in vessels treated by Percutaneous Transluminal Coronary Angioplasty (PTCA) and several trials have been performed to investigate this hypothesis. Here again, it appears that pioglitazone and rosiglitazone behave differently. A meta-analysis by Geng et al. [72] including eight trials (six with pioglitazone and two with rosiglitazone) showed that thiazolidinedione therapy for 6 months after coronary stenting significantly reduced the risk of in-stent restenosis, the stenosis diameter, the late lumen loss and the neointimal area/volume ratio in both diabetic and non-diabetic patients. Another meta-analysis by Nishio et al., however, found that a significant decrease in the risk of target vessel revascularization following percutaneous coronary intervention was associated with the use of pioglitazone, but not of rosiglitazone [73]. The lack of any protective effect by rosiglitazone in terms of in-stent restenosis was also shown by the APPROACH trial, demonstrating no additional advantage in the use of rosiglitazone for reduction of in-stent restenosis in people with type 2 diabetes [74].

As to the possible mechanisms underlying the anti-restenosis effects of thiazolidinediones, Hong et al. [75] showed that the reduced neointimal hyperplasia within the stented lesion observed with pioglitazone treatment was preceded by a reduction in circulating natural killer cells and in IL-6 and monocyte chemo-attractant protein-1 levels, by downregulation of chemokine receptor 2 and by increased Interleukin-10 circulating levels. Furthermore, proliferation and migration of vascular smooth muscle cells were inhibited in the presence of pioglitazone-treated patient serum, demonstrating that the antiproliferative effects of pioglitazone occurred concurrently with its anti-inflammatory action.

Effects on the Liver

Insulin resistance is among the main culprits for the occurrence of Non Alcoholic Steato-Hepatitis (NASH), so that insulin resistance represents a logical therapeutic target for this disease [76]. In the PIVENS (Pioglitazone, Vitamin E or placebo for Nonalcoholic Steatohepatitis) trial [77], the largest trial completed to date on the role of thiazolidinediones in patients with histologically proven NASH and without diabetes or cirrhosis, pioglitazone use was associated with highly significant reductions in steatosis, inflammation and hepatocellular ballooning. Insulin resistance and liver enzymes improved as well and, as compared to placebo treated subjects, a greater proportion of patients receiving pioglitazone had complete resolution of steatohepatitis by the end-of-treatment biopsy (42 vs. 27%) [77]. Different meta-analyses have shown that, as compared to control therapy, glitazones significantly improved serum alanine aminotransferases as well as metabolic and histological variables [76,78,79]. The benefits of pioglitazone therapy on NASH were evident in both diabetic and non-diabetic patients: they were however more pronounced in non-diabetic patients [78]. In contrast to clinical trials with pioglitazone, results from clinical trials with rosiglitazone have been less encouraging. The FLIRT (Fatty Liver Improvement with Rosiglitazone Therapy) trial examined rosiglitazone or placebo in 63 patients without dietary intervention [80]. Among histologic outcomes, only steatosis was significantly improved after 1 year of treatment. Moreover, in the patients completing the 2-year FLIRT trial extension, notwithstanding continued improvement in insulin sensitivity and aminotransferases, rosiglitazone did not further improve liver histology [81].

The available data support the use of pioglitazone in patients with biopsy-confirmed NASH, particularly those with advanced NASH, who are at the highest risk of liver-related morbidity and mortality. However, since only a subset of NASH patients seems to respond favourably to pioglitazone treatment, pioglitazone cannot be considered the ultimate therapy for this condition. NASH, indeed, like other complex metabolic diseases, will very likely call for a multifaceted approach [76].

Thiazolidinediones: The Shortcomings

Weight Gain

A weight gain between 2 and 5% is a common side effect of thiazolidinedione treatment [82] and is more marked when these drugs are used in combination with sulfonylureas or insulin [83]. The mechanism for this weight gain is far from being fully understood. Some studies have reported that it is mostly because of fluid retention [84], others that it is largely [85] or almost totally [86] due to fat deposition. In any case, it is paralleled by a redistribution of adipose tissue from visceral to subcutaneous deposits [87] and is closely related to the efficiency of thiazolidinedione therapy in improving glucose control [88]. Since the weight gain is limited and coupled to favourable fat tissue redistribution, it is likely to have very little, if any, impact on cardiovascular risk. This may partly explain why, in the PROactive study, mortality actually decreased in the patients gaining weight on pioglitazone treatment [57].

However, weight gain might seriously impair patient compliance with treatment and, as such, does represent a limitation to the clinical use of thiazolidinediones.

Fluid Retention and Heart Failure

Lower extremities oedema occurs in about 7% of patients treated by thiazolidinediones in monotherapy and is even more prevalent (up to 15%) when insulin is used as co-treatment [89]. This is the result of a fluid retention which also contributes to lowering the hematocrit [90]. The mechanisms behind the thiazolidinedione-induced fluid expansion are not clearly understood. It has been thought that thiazolidinediones upregulate the expression and stimulate the translocation of the collecting duct epithelial sodium channel [90], but recent

data on mice challenge this hypothesis [91]. The fluid retention is often mild and can be dealt with clinically [92], but it might be behind the increased risk of macular oedema reported in predisposed patients with the use of thiazolidinediones [93]. In this regard, it should be noted that significantly increased odds of macular oedema with the use of thiazolidinediones (both rosi- and pioglitazone) were reported in a retrospective study based on a UK cohort [94] and emerged as well from the analysis of the Kaiser Permanente database in Southern California [95]. An analysis conducted on the US Food and Drug Administration Adverse Event Reporting System (FDA-AERS) database [96] also supported the possibility of an increased macular oedema risk associated with the use of thiazolidinediones. On the other hand, clinical trials such as the ACCORD Eye Study [97] or the RECORD study [98] and the PROactive [57] failed to show any increased risk of macular oedema associated with the use of either thiazolidinedione.

Fluid retention is likely to exert a key role in the pathogenesis of heart failure associated with the use of thiazolidinediones. In the RECORD study, the risk of heart-failure-related hospitalization and death was doubled in subjects treated with rosiglitazone [99]. Furthermore, the same meta-analysis showing a reduction in the risk of a composite cardiovascular endpoint associated with the use of pioglitazone, also showed a significant increase in the risk of serious heart failure [52]. In the PROactive study, 5.7% pioglitazone-treated patients were reported with serious heart failure, compared with 4.1% of placebo-treated patients: however, rates of heart-failure-related mortality were similar in both groups [100,101]. Moreover, a *post hoc* time-to-event analysis showed that, among patients with serious heart failure, the risk of subsequent death, MI or stroke was 36% lower in the pioglitazone group than placebo [100]. Finally, an observational, retrospective analysis of a Medicare database relating to more than 200 000 patients aged 65 or older, showed that prescription of rosiglitazone was associated, unlike pioglitazone, with a significant 25% increase in the risk of reported heart failure [102]. It thus appears that in terms of the risk of heart failure pioglitazone again has a more favourable profile than rosiglitazone. Nevertheless, cardiac function needs to be closely monitored in pioglitazone-treated patients and patients at risk of heart failure should not be put on pioglitazone. This of course, is a further limitation on pioglitazone use.

Bone Fractures

A suggestion of increase in bone fractures in women associated with the use of both rosiglitazone and pioglitazone already emerged from the large intervention trials ADOPT [18] and PROactive [101].

More recently, Habib et al. [103] showed that thiazolidinedione use was associated with an increased risk of fractures in women, particularly at ages above 65 years, but not in men. Similar results were obtained by Dormuth et al. [104] but, interestingly, in this study pioglitazone was associated with a greater risk than rosiglitazone, and, as opposed to rosiglitazone, affected the risk of fracture in men as well as in women. It was calculated that it takes 86 subjects treated with either thiazolidinedione for 3 years before an additional peripheral fracture will occur as compared to sulfonylurea-treated patients [104]. A similar NNH (111 subjects/3 years) for peripheral fracture risk can be calculated from a review of 19 unpublished studies relating to over 8000 patients treated with pioglitazone [105]. In this review however, the risk was limited to women and no difference in bone fracture prevalence was observed between exposed and non-exposed men. A significant increase in fracture risk in women but not in men with the use of either rosiglitazone or pioglitazone resulted also from a meta-analysis analyzing data from 10 randomized controlled trials involving over 13 000 subjects and two observational studies relating to 31 679 subjects [106]. In two of the randomized trials where it was measured, bone mineral density in women exposed to thiazolidinediones was significantly reduced at the lumbar spine and at the hip [107,108].

Again, in a Scottish cohort of people with type 2 diabetes, thiazolidinedione treatment was associated with an 18% relative increase in hip fracture rates for every cumulative year of thiazolidinedione exposure, in both men and women. The risk was similar for rosiglitazone and pioglitazone [109]. The mechanisms for decreased bone density associated with thiazolidinedione use are not completely understood. Adipocytes and osteoblasts come from a common progenitor upon activation of specific transcription factors. Activation of Runx-2 drives progenitors to become osteoblasts while activation of PPAR-γ results in adipocyte differentiation [110]. As thiazolidinediones are PPAR-γ agonists, it has been postulated that thiazolidinediones may increase adipocyte differentiation at the expense of osteoblasts *in vitro* [111,112]. However, in the adult skeleton osteoblasts represent less than 10% of the overall cell population which is mainly composed of osteocytes [110]. *In vitro* data have recently shown that thiazolidinediones might induce osteocyte apoptosis [113], probably through a GPR40-dependent mechanism [110]. In summary the increased bone fracture risk does represent a limitation on thiazolidinedione use and weighs heavily against the benefits of thiazolidinedione therapy in women and especially in women of menopausal age, as the large majority of T2DM women tend to be. Caution must therefore be used in prescribing a thiazolidinedione for a menopausal woman presenting one or more of the risk factors listed in Table 1.

Cancer

In the month of July 2011 the European Medicines Agency issued a press release stating that evidence was available showing a small increase in bladder cancer risk associated with pioglitazone treatment in male patients.[b] Such evidence was gathered from epidemiological studies [114–116] pointing to a relative risk ranging from 1.12 to 1.33 in pioglitazone-exposed diabetic patients, in particular patients treated for the longest duration and with the highest cumulative doses. Furthermore, in a meta-analysis of randomized controlled clinical studies, 0.15% of pioglitazone treated subjects (19 out of 12 506) had bladder cancer versus 0.07% (7 out of

[b]European Medicines Agency, (2011) Questions and answers on the review of pioglitazone-containing medicines (Actos, Glustin, Competact, Glubrava and Tandemact). http://www.ema.europa.eu/docs/en_GB/documentlibrary/Medicine_QA/2011/07/WC500109179.pdf.

Table 1. Factors identifying people who should be assessed for osteoporosis before starting pioglitazone treatment. Adapted with permission from Ref. [127].

Major risk factors	Minor risk factors
Vertebral compression fracture	Rheumatoid arthritis
Fragility fracture after age 40	Past history of clinical Hyperthyroidism
Family history of osteoporosis fracture (especially maternal hip fracture)	Long-term anticonvulsant therapy
Systemic glucocorticoid therapy of > 3 months' duration	Low dietary calcium intake
Malabsorption syndrome	Smoker
Primary hyperparathyroidism	Excessive alcohol intake
Propensity to fall	Excessive caffeine intake
Osteopenia apparent on X-ray film	Weight < 57 kg
Hypogonadism	Chronic heparin therapy
Early menopause (before age 45)	

10 212) of subjects not exposed to the drug [117]. It should be noted, however, that the strongest data pointing towards an association between pioglitazone use and cancer were gathered by French population-based study. In that cohort no significant association was observed between pioglitazone exposure and the incidence of lung cancer, colorectal cancer, breast cancer in women or kidney cancer. For head and neck cancer, the adjusted hazard ratio was 0.85 [95% CI 0.73, 0.99]; $p = 0.041$ [115]. The role of PPARγ activation in carcinogenesis is indeed still debated and probably differs according to the different cell types. Thus, PPARγ activation has been found associated with a decreased risk of cancer in tissues such as liver, colon, [118], lung and prostate [119]. *In vitro*, PPARγ agonists have been reported as protective against cancer development [120] or able to induce it [120]. Thus, in humans, when all cancers are considered, and particularly breast cancer, a trend has been shown suggesting pioglitazone is protective [121]. On the other hand, while pioglitazone was not genotoxic or carcinogenic in mice, bladder tumours were observed in male rats. The increased risk of bladder tumour was, however, eliminated in these animals following urine acidification, which prevented crystal formations [122]. Pioglitazone administration might directly induce bladder epithelium hyperplasia in the rat, on the other hand: it has been postulated that, on this substrate, the chronic irritation linked to crystal deposition might trigger metaplasia and favour cancer development [116]. It must be pointed out, however, that the absolute risk of bladder cancer remains low (10 620/year patients need to be treated before finding one bladder cancer[c]). In the EMA's own words: 'the benefits of pioglitazone continue to outweigh its risk in patients responding adequately to treatment: certain measures however, will need to be taken to reduce the risk of bladder cancer'.[b] These measures involve excluding from pioglitazone treatment patients with bladder cancer (present or anamnestic) or an uninvestigated history of macroscopic hematuria and implementing risk reduction strategies in all patients. These include warning patients of increased risk for bladder cancer, limiting pioglitazone use in the elderly, following up the patients at 3–6 month intervals, carefully defining efficacy targets and the time frame to achieve and maintain them (while stopping pioglitazone treatment if such targets are not achieved), starting with lower doses and only stepping up therapy if the lower doses do not achieve target.[b]

[c]Caisse Nationale de l'Assurance Maladie (2011) Risque de cancer de la vessie chez les personnes diabétiques traitées par pioglitazone en France: une étude de cohorte sur les données du SNIIRAM et du PMSI. (http://www.ameli.fr/l-assurance-maladie/statistiques-etpublications/pioglitazone-et-cancer-de-la-vessie.php).

Conclusions

Having briefly reviewed the advantages and shortcomings of pioglitazone treatment, one is left with the problem of assessing their relative weight and calculating their 'algebraic sum'. Of course, only if this 'sum' turns out to be positive could it be concluded that pioglitazone still has a role in the T2DM treating algorithm. Several factors, however, affect the way the scale is going to tilt (figure 1). One has to consider the availability, efficacy and safety of alternative treatments, as well as the possibility of combining pioglitazone with one or more of them. Finally, and most important, one has to consider each patient's peculiar characteristics, since, as we will discuss, it is according to these that pioglitazone benefits might outweigh its hazards or vice versa.

If blood glucose reduction were the only reason for using an anti-diabetes medication, then with the advent of other treatments with more or less the same blood glucose lowering efficacy, but better tolerability (DPP-IV inhibitors, SGLT2 receptor antagonists, GLP-1 receptor agonists) [4,123], one would be hard pressed to keep using pioglitazone. However, in order to appreciate the 'weight' of pioglitazone's advantages, one has to consider that, with the progressively increasing knowledge of T2DM pathophysiology, we ought to have the ambition of treating the disease and modifying its course rather than just lowering blood glucose. This might be accomplished by targeting the disease's most likely pathogenic mechanisms. Impaired β-cell function, inappropriate glucagon secretion, altered adipocyte metabolism, increased hepatic glucose production, decreased insulin-dependent glucose uptake, altered incretin hormone secretion/action, increased kidney glucose reabsorption and altered brain nutrient sensing are the major defects leading to T2DM [124]. As discussed above, pioglitazone improves the adipocyte metabolism, has direct and indirect protective effects on the β-cell, improves insulin-dependent glucose uptake and, probably by reducing circulating FFA, helps contain hepatic glucose production [4]. Thus, directly or indirectly, pioglitazone positively affects four out of the eight major mechanisms thought to be responsible for T2DM occurrence and progression. As discussed above, not for nothing are thiazolidinediones the presently available diabetes treatment with the longest demonstrated 'durability' [14,16,17].

Thiazolidinediones are also a class of drugs which have been shown most effectively to prevent conversion of impaired

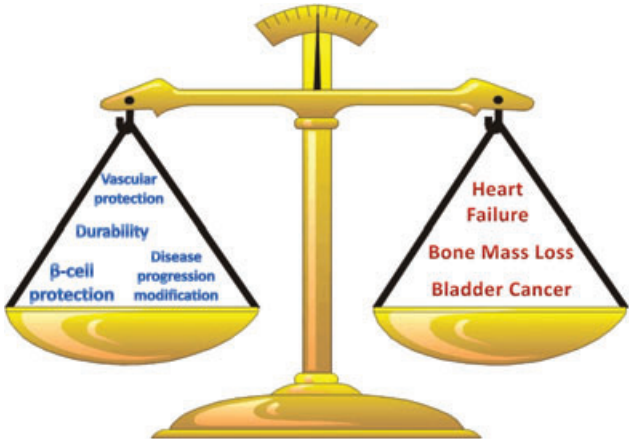

Figure 1. Benefits and hazards of pioglitazone therapy.

glucose regulation (IGF and/or IGT) into overt T2DM [125]. Besides, T2DM being a multifactorial disease, it would be wise to use a combination of different molecules aimed at different targets to treat it, very much as is currently done in treating hypertension [4]. It is not been proven in clinical trials but, theoretically, combining pioglitazone with metformin and either a GLP-1 receptor agonist or a DPP-IV inhibitor in the early phase of the disease (as proposed by Ralph De Fronzo [125]) might have a strong impact in modifying disease progression. By this approach, in fact, 6 out of the 8 major mechanisms thought to be responsible for T2DM will be targeted by one, two or three drugs (see figure 2). Finally, the ideal anti-diabetes agent should have a positive impact on cardiovascular risk. A large body of pre-clinical data suggests indeed that incretin-based therapies might have a protective effect towards cardiovascular disease, regardless of glucose control. Clinical trials aimed at verifying whether this is true are in progress, but data will not be available for at least another couple of years. Pioglitazone is the only anti-diabetes drug for which a study specifically designed for the purpose (PROactive study) has documented a significant association with reduced cardiovascular risk [57]. This is another very good reason for keeping pioglitazone in the T2DM treatment algorithm. It is true that a peculiar population of very high risk subjects with diabetes

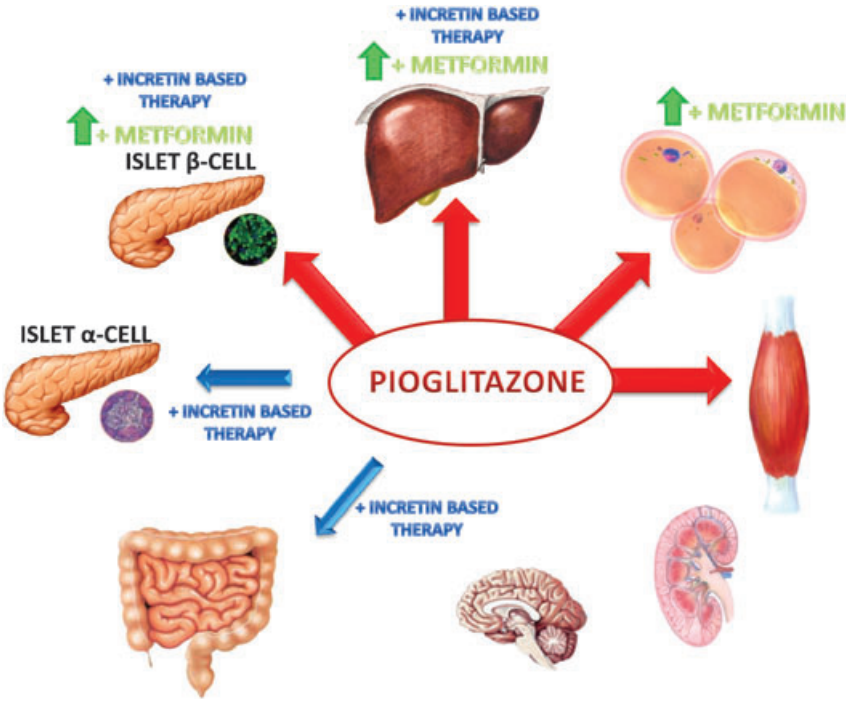

Figure 2. Key defects implicated in type 2 diabetes pathophysiology as a potential target of different treatment strategies.

was recruited in the PROactive study. A large, sulphonylurea-controlled, randomized trial in people with type 2 diabetes without previous cardiovascular disease is presently in progress, to observe whether the positive cardiovascular effects of pioglitazone therapy can also be demonstrated in this population.[d]

Notwithstanding all of the above, potentially dangerous side effects do present limitations to pioglitazone use. These limitations might be more severe than with other drugs. This is why it is extremely important to identify subjects with the best risk/benefit profile and use pioglitazone mostly on them. It is fairly easy to identify the best and the worst candidate respectively for pioglitazone treatment. The ideal candidate would be a middle-aged male, with a relative short disease duration, body mass index above 30 kg/m^2, increased waist circumference, affected by fatty liver disease (NAFLD), good left ventricle ejection fraction, a history of vascular disease, no signs or history of bladder cancer. Fluid retention and bone fractures should not be an issue in this subject, the increased abdominal fat deposit is the perfect target for pioglitazone action, the low or absent risk of hypoglycaemia makes it easier to conduct an active life and pioglitazone might help prevent future cardiovascular events. Should metformin and/or an incretin-based drugs be used in combination, this might help to curb the weight increase. On the other hand, the worst possible candidate would be an older woman, with long-standing disease, lean, with reduced bone mass and possibly with initial heart failure. In such an individual, the hazards associated with pioglitazone therapy will by far exceed the benefits.

Unfortunately, those described above are clear-cut examples standing at opposite extremes. In the middle, there are millions of people with type 2 diabetes in whom it would be far more difficult to decide whether pioglitazone therapy is worth the hazards that it might entail. It is our opinion that the pioglitazone benefit/risk ratio would still be positive in a large enough number of them, especially considering that the NNH for some of the most worrisome side effects such as cardiac failure and bladder cancer is high, as demonstrated in a very recent meta-analysis of pioglitazone studies (Edoardo Mannucci, personal communication). Nevertheless, scrupulous clinical judgment must be used whenever pioglitazone is prescribed. Clinical judgment is based on knowledge but, more than anything else, on expertise. Quoting E. Gale: '. . . expertise, being unmeasurable, will always be undervalued. It ranks with health and happiness as something never fully appreciated until absent, as when you witness the damage that lack of expertise can inflict upon other people's lives . . .' [126], although undervalued, expertise is exactly what is needed if we are to continue to use pioglitazone, as we think appropriate, in the treatment of T2DM, at least until newer PPARγ agonists or PPARγ modulators are devised able to retain the positive effects of pioglitazone with less adverse effects and greater tolerability.

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[d]Tosca.it.

Conflict of Interest

A. C. has received fees for lectures and/or participation in Advisory Boards from AstraZeneca, Bristol Mayer Squibb, Boehringer Ingelheim, Eli Lilly, Merck Sharp & Dhome, Novartis, Novo Nordisk, Sanofi-Aventis and Takeda. This study was designed by A. C. and G. F.

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