# Pleiotropic effects of glitazones: a double edge sword?

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Glitazones (thiazolidinediones) are drugs used for diabetes mellitus type 2. By binding to peroxisome proliferator-activated receptor γ (PPARγ) they modulate transcription of genes of carbohydrate and lipid metabolism. Through PPARγ stimulation, however, glitazones also affect other genes, encompassing inflammation, cell growth and differentiation, angiogenesis, which broads their therapeutic potential. The gene expression profile induced by each glitazone shows peculiarities, which may affect its benefit/risk balance; indeed, troglitazone and rosiglitazone have been associated with liver failure and coronary disease, respectively; whether or not these severe adverse effects are solely related to PPARγ remains yet unclear, since glitazones exert also PPARγ-independent effects. Glitazone chemistry serves as scaffold for synthesizing new compounds with PPARγ-independent pharmacological properties and we report here a preliminary observation of inhibition of vasoconstriction by troglitazone in isolated vessels, an effect that appears fast, reversible, and PPARγ-independent. Pleiotropic effects of glitazones need specific attention in terms of drug safety, but also provide basis for drug development and novel experimental therapeutics.
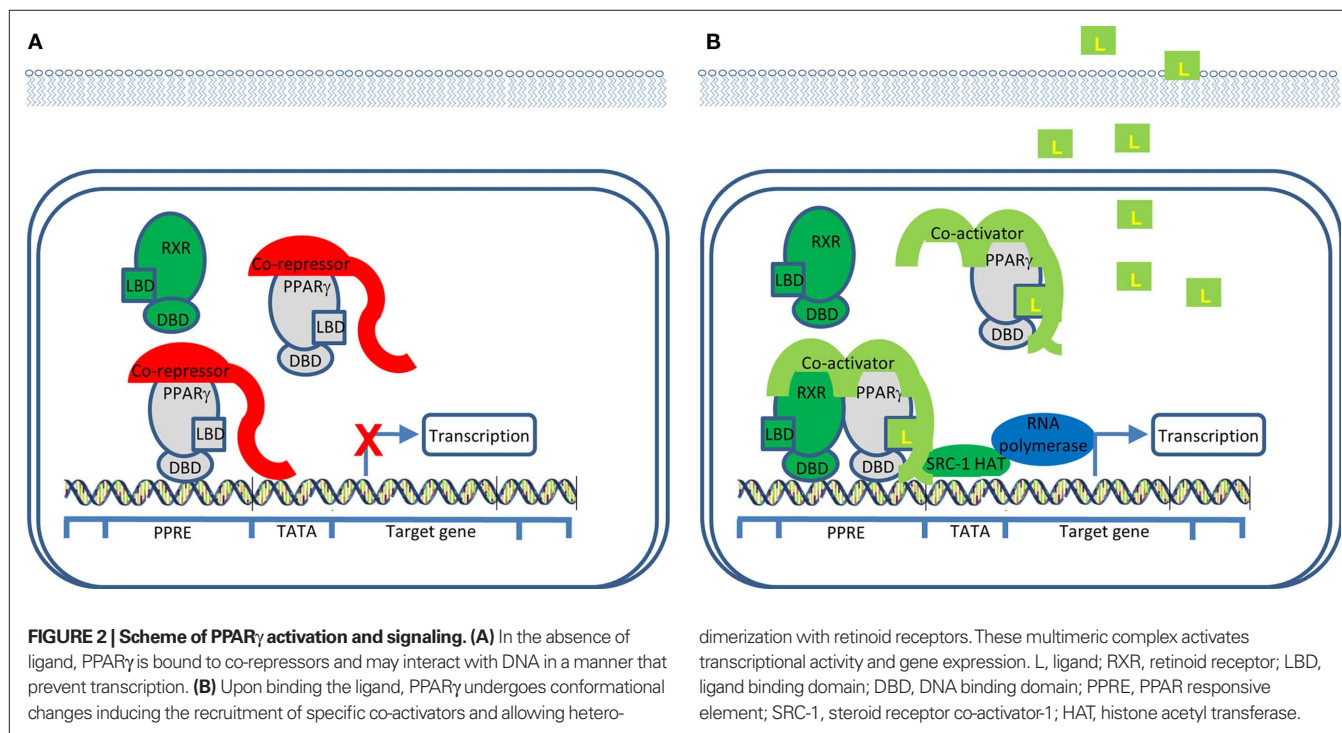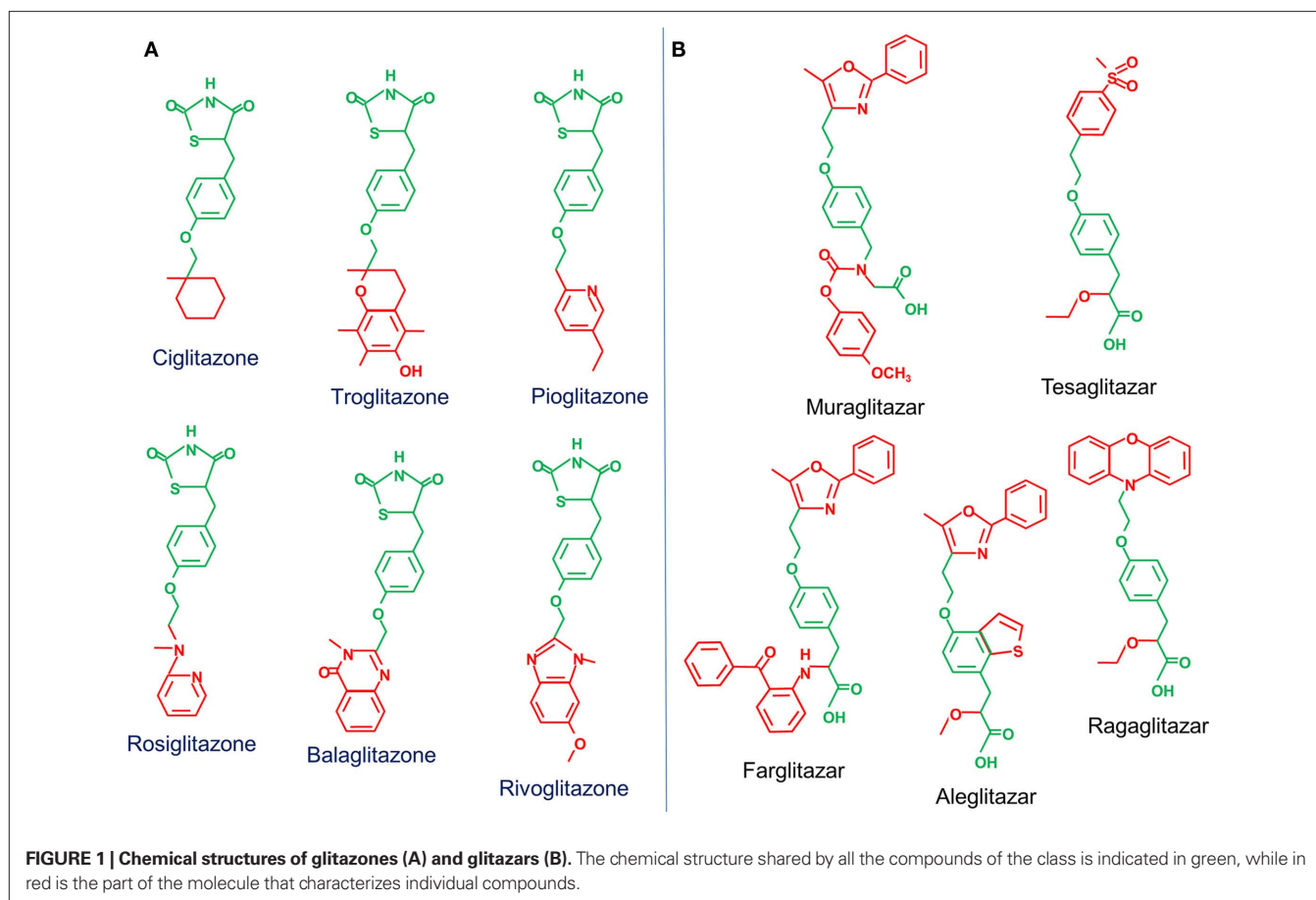
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Glitazones (also referred to as thiazolidinediones) are drugs approved for use in the treatment of diabetes mellitus type 2; they include ciglitazone, pioglitazone, troglitazone, rosiglitazone, rivoglitazone, and balaglitazone. Despite their striking chemical similarity (**Figure 1A**), these compounds have different safety profiles, such that only pioglitazone is currently still in clinical use; ciglitazone never reached the market, troglitazone and more recently rosiglitazone have been withdrawn (rosiglitazone is still sold in United States, but put under restriction), while rivoglitazone and balaglitazone are still in development. After being introduced in the 1990s, glitazones became very popular and widely prescribed, because they increase insulin sensitivity without causing hypoglycemia, until some clinical studies raised concerns on their safety (see below). In 2009 pioglitazone was selling for about 2.5 billion $ in United States, ranking ninth among top selling drugs (Drugs.com[1]). Glitazones act by binding to peroxisome proliferator-activated receptor γ (PPARγ), a nuclear receptor involved in regulation of insulin sensitivity and glucose metabolism (Francis et al., 2003). Following activation by exogenous ligands, such as glitazones, or endogenous ligands, such as free fatty acids and eicosanoids, PPARγ modulates transcription of genes involved in carbohydrate and lipid metabolism (**Figure 2**; Francis et al., 2003). PPARγ-dependent effects of glitazones include decrease of insulin resistance (Fujita et al., 1983), induction of adipocyte differentiation (Kletzien et al., 1992), inhibition of vascular endothelial growth factor (VEGF)-induced angiogenesis (Panigrahy et al., 2002), change in levels of leptin (De Vos et al., 1996) and adiponectin (Yamauchi et al., 2001), decrease in levels of some cytokines (Jiang et al., 1998; Ricote et al., 1998), including tumor necrosis factor α (TNFα), and interleukin-6 (IL-6; Sigrist et al., 2000), though this latter effect may

also be, in part, PPARγ-independent (see below). Glitazones also upregulate the expression of genes involved in fatty acid uptake, beta-oxidation, electron transport, and oxidative phosphorylation in subcutaneous fat (Boden et al., 2005), which may reduce plasma levels of lipids. Glitazones induce a moderate decrease in triglycerides and free fatty acids and an increase in high-density lipoprotein (HDL) cholesterol, while increasing the size/decreasing the density of low-density lipoprotein (LDL; Goldberg, 2006). This list of effects induced by glitazones is far from being complete, but may give an idea of the multiplicity of genes regulated following PPARγ stimulation; these genes go beyond glucose and lipid metabolism, encompassing inflammation (cytokines), cell growth and differentiation, angiogenesis (VEGF), which provides the basis for additional potential therapeutic indications. In fact, beside diabetes, glitazones have been investigated in a number of diseases, such as non-alcoholic steatohepatitis (Neuschwander-Tetri et al., 2003), psoriasis (Ellis et al., 2000), autism (Boris et al., 2007), polycystic ovary syndrome (Katsiki et al., 2009), and other conditions, potentially including also breast carcinoma (Baranova, 2008).

Some consequences of PPARγ stimulation, however, may not be beneficial or may even be harmful. Gene expression changes induced by glitazones are likely to be different in different cell types. *In vitro* studies with hepatocytes have shown that the gene expression profiles following troglitazone and ciglitazone exposure are clearly distinguished from those following pioglitazone and rosiglitazone. Genes that are differentially expressed between the more toxic troglitazone/ciglitazone group and the less toxic rosiglitazone/pioglitazone group are involved in necrotic, apoptotic, and cell proliferative pathways (Guo et al., 2006). Troglitazone seems to be more potent than other glitazones in inducing genes related to oxidative stress, such as heme oxygenase 1, or genes involved in DNA repair and cell death, such as

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[1]http://www.drugs.com/top200.html

**FIGURE 1 | Chemical structures of glitazones (A) and glitazars (B).** The chemical structure shared by all the compounds of the class is indicated in green, while in red is the part of the molecule that characterizes individual compounds.



**FIGURE 2 | Scheme of PPARγ activation and signaling. (A)** In the absence of ligand, PPARγ is bound to co-repressors and may interact with DNA in a manner that prevent transcription. **(B)** Upon binding the ligand, PPARγ undergoes conformational changes inducing the recruitment of specific co-activators and allowing hetero-dimerization with retinoid receptors. These multimeric complex activates transcriptional activity and gene expression. L, ligand; RXR, retinoid receptor; LBD, ligand binding domain; DBD, DNA binding domain; PPRE, PPAR responsive element; SRC-1, steroid receptor co-activator-1; HAT, histone acetyl transferase.

Gadd45 (Vansant et al., 2006). The withdrawal of troglitazone has led to concerns also on the other glitazones about their potential to induce liver failure (approximately 1 in 20,000 with troglitazone). Because of this, Food and Drug Administration (FDA) recommends 2–3 months checks of liver enzymes for the first year of glitazone therapy for early detection of this rare but very severe adverse effect. To date, with the newer glitazones, rosiglitazone, and pioglitazone, reported liver toxicity is much less frequent and severe. Liver toxicity has also been attributed to metabolic transformation of troglitazone by cytochrome P450 (CYP) 3A with generation of a quinone metabolite (He et al., 2001); however, no correlation between generation of the reactive metabolites and susceptibility to the troglitazone cytotoxicity has been demonstrated, while chemical inhibitors of drug metabolizing enzymes do not protect the cells against the toxicity (Masubuchi, 2006). The precise molecular mechanism of glitazone-induced liver injury remains to be determined; however, based on troglitazone-induced expression profile, which includes oxidative-apoptotic genes, on the mechanism of liver injury, that involves mitochondrial damage, with potential release of cytochrome *c* (Smith, 2003; Lee et al., 2008), on the pro-inflammatory effects of PPARγ ligands on hepatic stellate cells (Marra et al., 2000), we may hypothesize that PPARγ receptor stimulation may take part into the mechanism of hepatic injury. Worthy of note, troglitazone-induced gene expression profile exhibit high variability in individuals (Rogue et al., 2010), which may provide the basis for idiosyncratic reactions; genetic polymorphisms responsible for such reactions remain to be elucidated, but may include both genes involved in drug metabolism (CYP isoforms) and in PPARγ activity (PPAR co-activators, co-repressors, etc.).

A common side effect of all glitazones is water retention, which represents a significant risk in patients with decreased ventricular function. Increasing sodium retention and plasma volume expansion have been related to PPARγ stimulation in the epithelium of the renal collecting duct (Chen et al., 2005). There is some thought that amiloride or spironolactone could decrease this effect (Chen et al., 2005). Another adverse effect of glitazones related to PPARγ activation is on bone; glitazones decrease bone formation and bone mass, and increase fracture rates, at least in women (Bodmer et al., 2009). PPARγ inhibits bone formation by diverting mesenchymal stem cells from the osteogenic to the adipocytic lineage and increases bone resorption by inducing the development of osteoclasts. Other indirect mechanisms may involve levels of hormones and cytokines that affect bone metabolism (Bodmer et al., 2009).

An increased risk of coronary heart disease has been observed with rosiglitazone (Nissen and Wolski, 2007; Kaul et al., 2010). Recent evidence, however, suggests that rosiglitazone itself decreases the progression of atherosclerosis (Gerstein et al., 2010). In keeping with this, pioglitazone has been shown to afford significant protection from cardiovascular events in diabetic patients (Kaul et al., 2010). Protection against plaque progression seems therefore to be a class effect of glitazones, that might be related to PPARγ-dependent induction of adiponectin (Tao et al., 2010). At present, the precise mechanism(s) through which rosiglitazone increases the incidence of cardiac events is unknown and possibly unrelated to PPARγ stimulation.

The multiplicity and variety of glitazone effects through PPARγ stimulation has raised the question on whether different compounds exhibit different pharmacological profiles and/or a same compound differently affects PPARγ-dependent gene expression in different cell types. The actual view of nuclear receptor regulation by co-regulators, summarized in **Figure 2**, assumes that in the absence of ligand, the nuclear receptor binds to co-repressors with which it can be recruited on DNA to prevent transcription. Upon binding of endogenous or exogenous ligands, the ligand binding domain of PPARγ may undergo conformational changes inducing the recruitment of specific co-activators. These multimeric complexes determine transcriptional activity by bridging transcription factors to the basic transcription machinery and by specifically modifying chromatin structure (Gelman et al., 2007). Development of more selective PPARγ ligands, that induce recruitment of specific co-regulators, may implement the beneficial pharmacological actions of glitazones over their adverse effects (Gelman et al., 2007). However, because activation of other nuclear receptors, such as PPARα, exerts also beneficial metabolic and cardiovascular actions, the opposite strategy of broadening the pharmacology of PPAR-interacting compounds is also exploited in drug discovery and development. This latter strategy has recently brought PPAR dual ligands, termed glitazars (Tenenbaum et al., 2005), interacting with both PPARα and PPARγ. These drugs include muraglitazar, tesaglitazar, ragaglitazar, farglitazar, aleglitazar (**Figure 1B**). Despite their promising effects, however, none of them has yet reached the market and some have been stopped during clinical development.

## PPARγ-UNRELATED EFFECTS OF GLITAZONES AND DRUG DISCOVERY

Compounds containing the glitazone/thiazolidinedione moiety have been synthesized and screened for binding to diverse molecular targets. As can be seen from the following list, some of this targets are protein kinases and phosphatases. In terms of *in vitro* analytical pharmacology, this implies that kinetics of PPARγ-independent responses to these compounds is expected to be faster (within minutes), than PPARγ-dependent gene expression-related effects (Sears et al., 2007).

Class I phosphoinositide 3-kinases (PI3Ks), in particular PI3Kγ, have become attractive drug targets of glitazone-related compounds as potential treatments for inflammatory and autoimmune conditions (Pomel et al., 2006). Importantly, PI3Kγ is also involved in cardiovascular patho-physiology (Alloatti et al., 2004; Oudit et al., 2004), in cardiomyocytes as well as in endothelium and vascular smooth muscle cells, through Akt/protein kinase B stimulation; cardiovascular diseases may therefore represent, in the near future, an additional therapeutic field for glitazone-related compounds. Beside PI3K signaling, other PPARγ-unrelated mechanisms of glitazones may find usefulness in inflammation: inhibition of macrophage/monocyte chemotaxis, which plays a significant role in acute liver injury (Luo et al., 2010); activation of adenosine monophosphate-activated protein kinase and suppression of IL-6 production (Guh et al., 2010); inhibition of autotaxin, an extracellular enzyme that hydrolyzes lysophosphatidylcholine to produce lysophosphatidic acid (Albers et al., 2010), thereby inducing cell proliferation and/or chemotaxis.

Cancer therapy is another potential field of glitazone-related compounds. Glitazones down-regulates cyclin D1 through proteasome-facilitated proteolysis (Huang et al., 2006); using glitazone structure as chemical scaffold, other inhibitors of cyclin D1 have
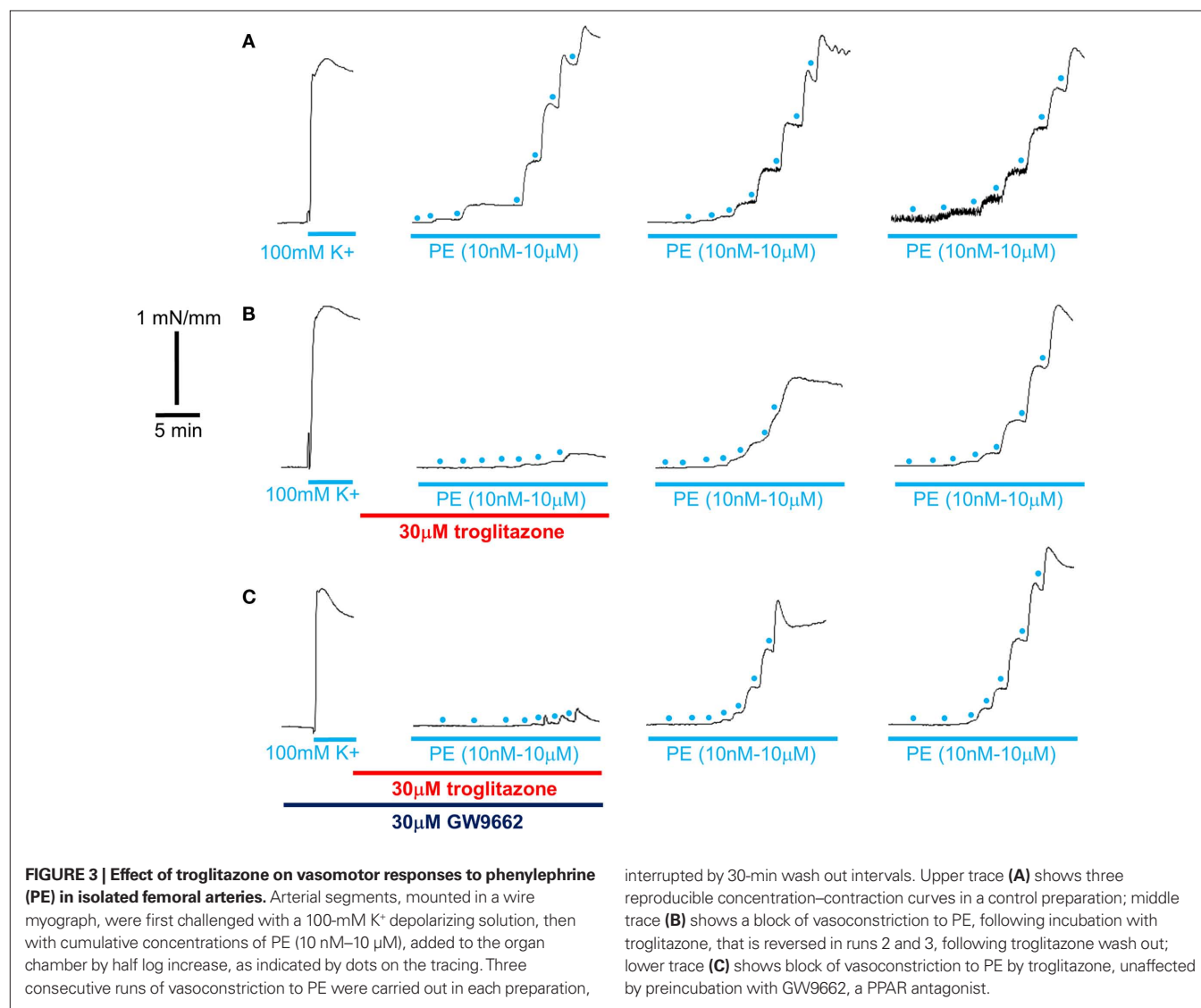
recently been designed and screened for potential anticancer activity (Colin et al., 2010). Increased proteasomal degradation by glitazones is not limited to cyclin D1, but includes important apoptosis regulatory proteins such as FLICE-inhibitory protein (FLIP) and beta-catenin, and the transcription factor Sp1 (Yang et al., 2008; Wei et al., 2009). Additional targets of glitazone-based drug design potentially exploitable for cancer therapy are: SHP-2, a tyrosine phosphatase that mediates cell signaling by growth factors and cytokines via the mitogen-activated protein kinase (MAPK) pathway (Geronikaki et al., 2008); Pim-1 and Pim-2 protein kinases frequently over-expressed in prostate cancer and certain forms of leukemia and lymphoma (Xia et al., 2009); Insulin-like growth factor-1 receptor, which may affect cell proliferation and survival (Liu et al., 2010).

## VASCULAR EFFECTS OF GLITAZONES

Inflammation is a key mechanism in the process of vascular athero-sclerosis. Plaque progression involves a number of mediators such as adhesion molecules, growth factors, chemokines, cytokines, and matrix metalloproteinases that lead to weaken the fibrous atherosclerotic cap

and eventually to rupture, platelet aggregation, and thrombosis. It is generally thought that PPARγ activation favorably affects these inflammatory components (Marx et al., 1998, 2002; Pasceri et al., 2000). As mentioned above, however, glitazones exert several anti-inflammatory effects also in a PPARγ-unrelated manner, which may further impact, at least theoretically, atherosclerosis progression, and plaque evolution. Glitazones have been shown to increase endothelial release of nitric oxide and expression of VEGF, and to decrease expression of endothelin-1; these are generally considered PPARγ-dependent effects and, overall, may contribute to the risk reduction for stroke and myocardial infarctions (McGuire and Inzucchi, 2008).

While studying vasomotor effects of telmisartan (Siarkos et al., 2011), an angiotensin receptor antagonist endowed with PPARγ agonistic activity (Schupp et al., 2004), we recently made the serendipitous observation that, *in vitro*, troglitazone rapidly and reversibly blocks contraction of vascular smooth muscle induced by either $K^+$-dependent depolarization or $\alpha_1$-adrenoceptor stimulation (**Figure 3**). Because this effect of troglitazone occurred already after only 30 min incubation, it does not seem to be consistent with



**FIGURE 3 | Effect of troglitazone on vasomotor responses to phenylephrine (PE) in isolated femoral arteries.** Arterial segments, mounted in a wire myograph, were first challenged with a 100-mM $K^+$ depolarizing solution, then with cumulative concentrations of PE (10 nM–10 μM), added to the organ chamber by half log increase, as indicated by dots on the tracing. Three consecutive runs of vasoconstriction to PE were carried out in each preparation, interrupted by 30-min wash out intervals. Upper trace **(A)** shows three reproducible concentration–contraction curves in a control preparation; middle trace **(B)** shows a block of vasoconstriction to PE, following incubation with troglitazone, that is reversed in runs 2 and 3, following troglitazone wash out; lower trace **(C)** shows block of vasoconstriction to PE by troglitazone, unaffected by preincubation with GW9662, a PPAR antagonist.

the latency of PPARγ-activated gene expression, which requires at least 2 h for significantly changing mRNAs (Sears et al., 2007). Furthermore, troglitazone-induced block of vasoconstriction was rapidly reversible, upon just 30 min wash out, which again makes unlikely PPARγ stimulation as the underlying mechanism, because reversibility would imply longer time related to the turnover of PPARγ-induced mRNAs and proteins; finally, the lack of effect of 30 μM GW9662, a PPARγ antagonist (Han et al., 2001) that we used at a concentration much higher than the reported IC50 (3.8 nM, Seimandi et al., 2005), rules out the involvement of PPARγ. At first sight, the concentration of troglitazone used in the present experiment, 30 μM, may look too high ("too" implying a plethora of potential non-specific effects); it is not that high, however, when considering that, *in vitro*, PPARγ stimulation by troglitazone is often tested at 20 μM (Rogue et al., 2010) and that in humans, troglitazone

$C_{max}$ is 1.5 μg/ml (corresponding to about 3 μM), following therapeutic 400 mg/day regimen (Loi et al., 1997). This preliminary observation, presently limited to just one glitazone, needs further investigation with other molecules of the class. Because this PPARγ-independent mechanism is likely to impact vascular tone *in vivo*, if confirmed with other therapeutically exploitable glitazones, such as pioglitazone, may have clinical significance in patients with type 2 diabetes, who are at high cardiovascular risk and/or have already developed cardiovascular diseases.

In conclusion, pleiotropic effects of glitazones need specific attention in terms of drug safety, but may provide basis for drug development and novel experimental therapeutics. Similar potential and risk may also apply to glitazars, molecules interacting with both PPARα and PPARγ, for which, at present, limited information is available.

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