

Two Faces of Pioglitazone: Sorting Out the Roles of its PPAR γ Binding Versus Mitochondrial Pyruvate Carrier Inhibition Is Not So Simple

Pioglitazone has been evaluated in multiple small clinical trials as a treatment for nonalcoholic steatohepatitis (NASH) with the rationale that its insulin-sensitizing effects, especially in adipose tissue, would be beneficial in NASH. Ligands for PPAR γ such as pioglitazone also induce adiponectin expression and secretion by adipose tissue and circulating adiponectin binds to receptors in the liver leading to increased β -oxidation of fatty acids, decreased gluconeogenesis and improved insulin sensitivity.⁽¹⁾

In the multi-center PIVENS trial of non-diabetic and non-cirrhotic subjects, pioglitazone 30 mg daily for 96 weeks improved the NAFLD activity score (NAS) compared to placebo.⁽²⁾ Following that, a single-center randomized clinical trial by Cusi et al.⁽³⁾ demonstrated that pioglitazone 45 mg daily for 18 months led to NASH resolution in 51% of patients with type 2 diabetes (vs 31% in placebo), along with reduction of hepatic triglyceride and improvement of insulin sensitivity in adipose tissue, liver, and skeletal muscle. These benefits were sustained over 36 months and the response was closely dependent on the pioglitazone concentration and exposure index.⁽⁴⁾

Interestingly, rosiglitazone, a 10-fold stronger PPAR γ agonist, does not cause similar improvement in NASH as pioglitazone. A meta-analysis⁽⁵⁾ of 8 trials using thiazolidinediones (TZDs) (5 using pioglitazone and 3 using rosiglitazone) combining a total 516 patients with biopsy-proven NASH, showed significant improvement in fibrosis of any stage by at least one stage (OR 1.66; 95% CI, 1.12-2.27) and advanced fibrosis by at least 2 stages (OR 3.15; 95% CI, 1.25-7.93). Similar improvements were seen in NASH patients without diabetes. In this pooled analysis, all improvements were attributed solely to pioglitazone, not to rosiglitazone, hinting that there may be more behind the effects of pioglitazone than its PPAR γ -binding properties.

However, weight gain and fluid retention are common side effects of PPAR γ agonists. An average of 2.5 to 4.8 Kg weight gain has been shown with treatment and up to 10% of patients develop leg edema. Although the weight gain is likely an on-target effect of PPAR γ signaling related to improved storage of lipid in subcutaneous adipose tissue and thus does not carry a metabolic risk, it is an undesirable side effect from a patient's perspective. Other dual- and pan-PPAR

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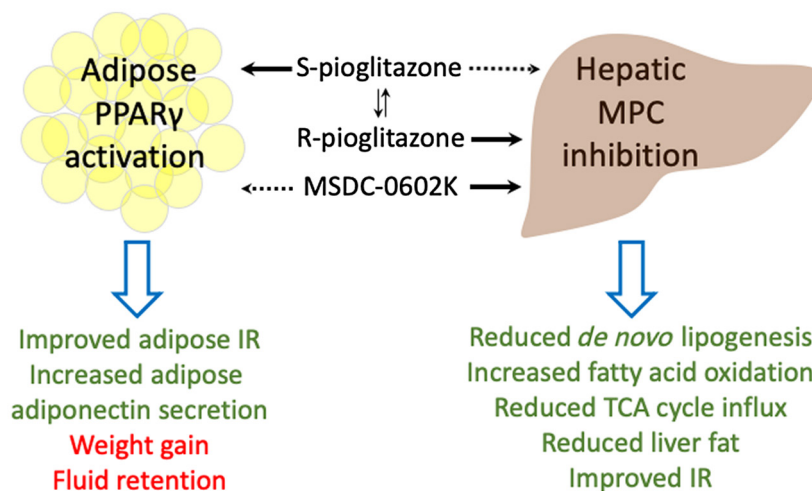


FIG. 1. The dual roles of pioglitazone: PPAR γ activation and MPC modulation.

agonists including saroglitazar (a PPAR α/γ agonist) and lanifibranor (a PPAR $\alpha/\delta/\gamma$ agonist) appear to cause roughly similar amounts of weight gain.

Recognizing these disadvantages of PPAR γ ligands and also the animal data showing an insulin-sensitizing effect of pioglitazone in mice with PPAR γ deletion, further work demonstrated that the TZDs such as pioglitazone are also inhibitors of the mitochondrial pyruvate carrier (MPC).^(6,7) Pyruvate is the product of glycolysis and enters mitochondria via the MPC where it can be converted to precursors for *de novo* lipogenesis. MSDC-0602K is a second-generation TZD designed to minimize direct binding to PPAR γ , but retain its ability to inhibit the MPC. When compared to pioglitazone, MSDC-0602K has very low affinity to PPAR γ ⁽⁸⁾ and its effect on NASH were recently evaluated in the EMINENCE trial. Unfortunately, it did not induce greater NASH resolution or fibrosis improvement compared to placebo.⁽⁹⁾ Despite minimal direct PPAR- γ activation, a dose-dependent increase in weight gain was observed between 62.5, 125, and 250 mg MSDC-0602K groups. While weight gain remains an issue with MSDC-0602K, human and animal data suggest that other TZD side-effects such as edema and bone-density loss were improved.^(9,10)

In this issue, Jacques et al., further explored the MPC-inhibiting properties of pioglitazone. Pioglitazone is a racemic mixture of two stereoisomers, R and S, based on one chiral carbon. The R and S forms interconvert due to relatively rapid hydrogen

exchange at that chiral center, but it was discovered that replacing the hydrogen with deuterium substantially slowed the interconversion and stabilized the S- or R-pioglitazone enantiomers. Using cell-free binding assays, they demonstrated that only the S form bound and activated PPAR γ , while both S and R enantiomers could inhibit the MPC. Therefore, to negate PPAR γ -agonism, they further developed and tested the deuterated R-pioglitazone, or PXL065, which was found to improve liver fat, inflammation, and fibrosis in mouse NASH models without significant weight gain or fluid retention. By comparison, deuterated-S-pioglitazone, the PPAR γ ligand, did not have these benefits in the liver yet it did cause weight gain and fluid retention.

Many questions remain to be answered. Whether these mouse studies translate into efficacy in human trials is of course the biggest challenge as it is with every therapy showing preclinical efficacy for NASH. But additionally, much more needs to be understood about the fate of biologically active pioglitazone metabolites such as their pharmacokinetics and pharmacodynamics. The pharmacokinetic analyses suggest that the deuterated enantiomers are not stable *in vivo*. When dosed with PXL065 (deuterated R-pioglitazone), a significant amount of both S- and R-pioglitazone that was protonated (no longer deuterated) were observed. With TZD dosing, the majority of what circulates is not the base compound, but several active PPAR γ -binding metabolites, known as metabolites-III (hydroxyl) and -IV (carbonyl) of pioglitazone. These metabolites were

measured in the dog and healthy human subject pharmacokinetic analyses, and notably the vast majority of the metabolites were protonated, not deuterated. In theory, without being deuterated, these metabolites revert to being a racemic mixture and capable of PPAR γ -binding. One benefit of PPAR γ ligands is the induction of adiponectin and interestingly, the studies of PXL065 demonstrated nearly equivalent increases in serum adiponectin levels after treatment with pioglitazone or deuterated S-pioglitazone as PXL065 (data shown in Supporting Fig. S5). Whether this is an indirect effect of the metabolic benefits of MPC inhibition or an effect of PPAR γ ligand activating metabolites of PXL065 remains to be shown. Ultimately, PLX065 needs to be tested in PPAR γ -/- animals and cell systems to ensure lack of PPAR γ -based pharmacology if the proposed mechanism of action is to be convincingly proven.

In summary, the recent study by Jacques et al leverages the ability to stabilize the R enantiomer of pioglitazone to study its effects on NASH in preclinical models. However, it is only partial stabilization and much more needs to be understood about its mechanisms of action. Moreover, it is still unclear if inhibition of the mitochondrial pyruvate carrier is a viable target in NASH. Since patients likely develop NASH by a variety of different mechanisms, targeting the MPC might be valuable in a subset of patients in whom the generation of precursors for de novo lipogenesis is driven by glucose excess and glycolysis. MPC inhibitors, like all insulin sensitizers, may be most beneficial in patients who are insulin resistant, but retain a degree of functional pancreatic beta cell mass for optimal pharmacology. Currently, our ability to subclassify or categorize NASH patients according to their underlying major drivers of disease remains elusive but as we expand our understanding of NASH, we may be able to match specific therapies to individual patients based on each person's underlying causes of NASH (Fig. 1).

Pioglitazone is a mixture of S and R enantiomers that interconvert. The S enantiomer is a PPAR γ ligand whereas the R enantiomer is a mitochondrial pyruvate carrier (MPC) inhibitor. PPAR γ ligands have been shown to be beneficial in some patients with NASH. MPC inhibitors may also have a role in treating NASH by decreasing hepatic de novo lipogenesis, increasing fatty acid disposal and decreasing TCA cycle influx. The drug MSDC-0602K was developed as a predominately MPC inhibitor and was recently evaluated in a phase 2 trial for NASH.

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