Novel Insulin Sensitizers Enhance Brown Adipose Cell Differentiation by Modulation of the Wnt Signaling Pathway

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Abstract:

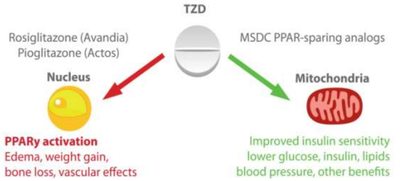
Brown adipose tissue (BAT) may play an important role in type 2 diabetes and obesity. Thiazolidinediones that do not bind to or activate PPARγ at pharmacological concentrations (PPARγ-sparing, PsTZDs) elicit differentiation of BAT progenitor cells through a mitochondrial target. MSDC-0160, a clinical PsTZD candidate, produced insulin sensitizing pharmacology without weight gain or plasma volume expansion in a Phase 2a clinical trial. To better understand how PsTZDs elicit differentiation of BAT, we investigated the signaling pathways that are engaged following drug treatment of cells. BAT progenitor cells isolated from mice exhibit enhanced mitochondrial biogenesis and expression of UCP1 four days after drug treatment although some changes in gene expression occurred within 12 hours. These actions were not blocked by PPARγ antagonists and occurred in cells null for PGC1α, demonstrating the PPARγ independent nature of the pathway. A decrease in mTOR activity was detected by 24 hours (prominently decreased at 48 hours). However, a simple inhibition of mTOR by the PsTZDs appears unlikely since rapamycin *inhibited* BAT differentiation. Since genetically enforced expression of Wnt signaling blocks BAT differentiation, we hypothesized that PsTZDs intercede in this pathway to permit progenitor cells to exit the cell cycle and undergo terminal differentiation. Wnt signal transduction was monitored by evaluating β-catenin and pGSK-3β (ser9) phosphorylation, followed by a substantial decrease in β-catenin, *before* the increase in UCP1. In contrast, pharmacologic enforcement of Wnt signaling with LiCl or a specific inhibitor of GSK-3β (Chi-99021) completely blocked PsTZD-induced changes in GSK-3β, β-catenin, *and* differentiation of BAT cells. Thus, the PPARγ-sparing mechanism produces terminal differentiation of BAT progenitor cells by modification of Wnt signaling, which includes an early reduction of GSK-3β phosphorylation linked to the PsTZD mitochondrial target. The elucidation of these signaling events may provide a path forward to new therapeutics independent of PPARγ activation.

Introduction:

In a Phase 2a study in type 2 diabetic patients, MSDC-0160:

Like pioglitazone:
Decreased glucose and insulin
Increased HDL cholesterol

But *unlike* pioglitazone:
Did not decrease circulating RBCs
Did not increase body weight



Methods:

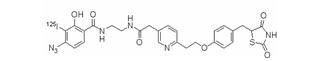
We have used the PsTZD clinical candidates, MSDC-0160 and MSDC-0602, to examine mitochondrial biogenesis in cells derived from brown adipose tissue (BAT). In this model system, BAT precursor cells are isolated from CD1 as well as PGC-1α knockout mice and maintained in culture (as described by Petrovic et al). At confluence, the precursor cells are treated with 25 nM insulin -/+ TZDs. We have measured expression of selective messages by RT-PCR and protein levels by Western blot. PPARγ binding activity was measured using a commercial FRET assay and interaction with the mitrochondrial target of the TZDs was measured by binding or crosslinking with a selective probe. Densitometry of Western blots and autoradiograms was conducted using ImageJ software. PGC-1α$^{-/-}$ mice were developed as reported by Leone et al (PLoS Biol 2005) and have been backcrossed into a pure C57BL/6 background.

References:

Petrovic N, et al. Am J Physiol Endocrinol Metab 295:E287-E296, 2008.
Leone TC, et al. PLOS Biology 3: 672-687, 2005.

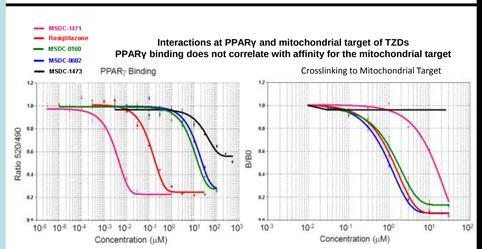
Results:

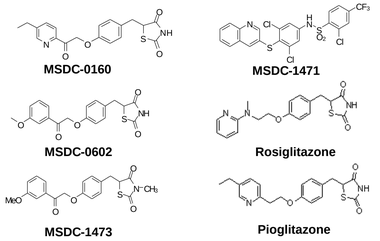
Photoaffinity crosslinker allows for identification of a specific mitochondrial membrane binding site for thiazolidinediones. Competition for crosslinking of mitochondrial target predicts activity in BAT cells.

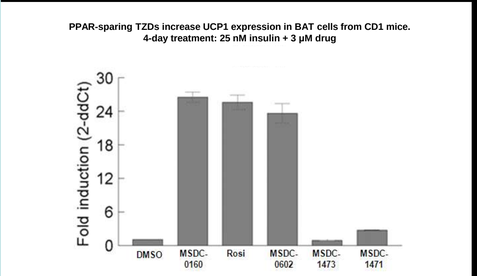


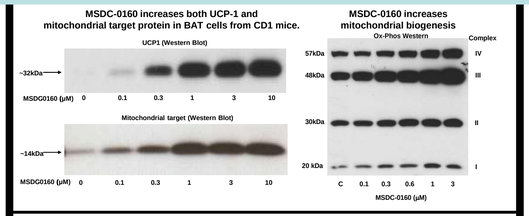
^{125}I crosslinking of mitochondrial target

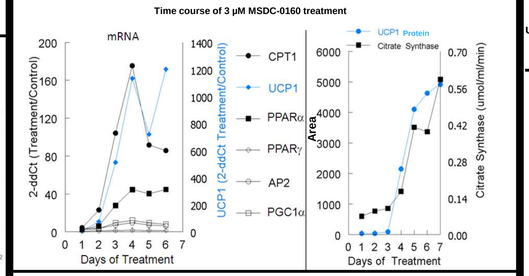
All compounds tested at 25 μM

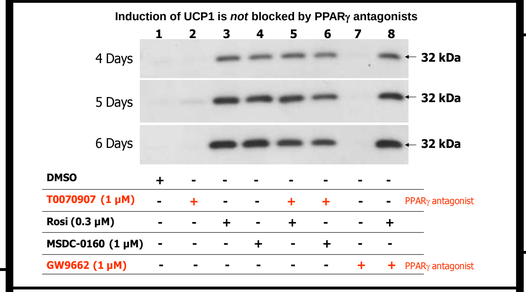


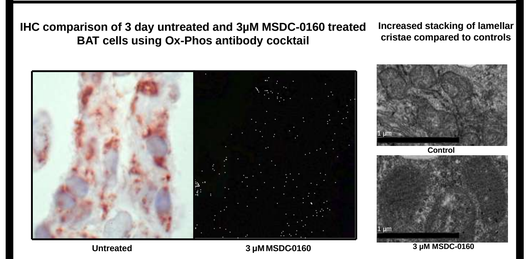


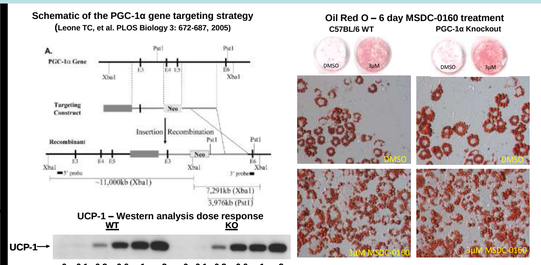


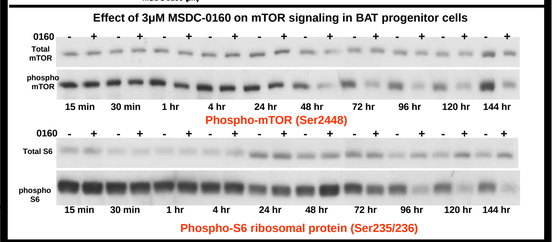


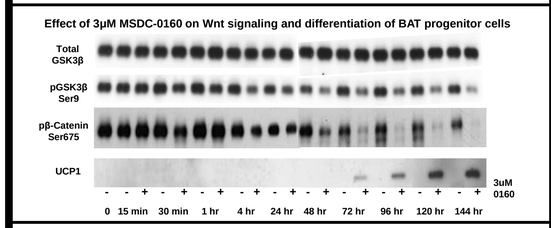


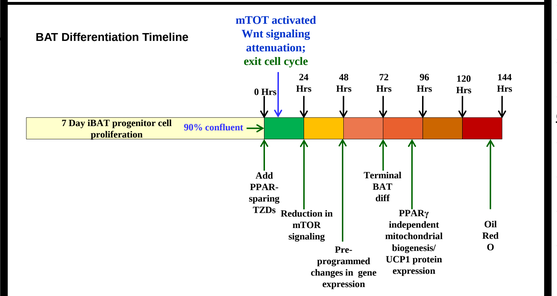




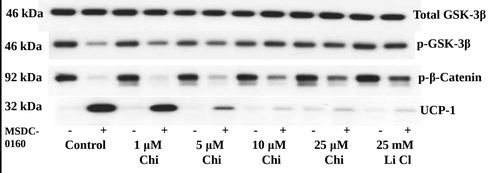


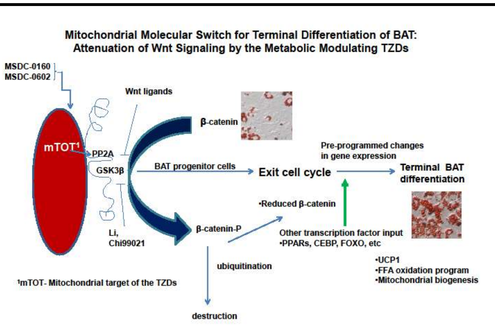






Enforcement of Wnt signaling with GSK3β inhibitors blocks PsTZD induced changes in GSK-3β phosphorylation, β-catenin, and BAT differentiation. Progenitor cells pre-treated with GSK3β inhibitor (Chi-99021 or LiCl) for 1 hour and then added DMSO or 1μM MSDC-0160 (cells received the inhibitor + drug for 24 hours). Total treatment time was 4 days.





Summary:

- MSDC compounds accelerate the differentiation of brown adipocytes, increase UCP-1 expression (message and protein), and increase mitochondrial biogenesis
- These effects of PPAR-sparing analogs are not mediated by PPARγ
  - No correlation with PPARγ activity among analogs
  - No effects of PPARγ antagonists
  - Correlation with binding and increased expression of the mitochondrial target
- These effects of MSDC-0160 are independent of PGC-1α expression and continue in knock out cells
- Prior to other changes in BAT precursor cells there is a modification of nutrient-sensing pathways with a reduction in mTOR signaling
- PPAR-sparing TZDs interact with mTOT to form a mitochondrial molecular switch that attenuates Wnt signaling
- This attenuation of Wnt causes the BAT progenitor cells to exit the cell cycle resulting in terminal differentiation of BAT
- Chemical enforcement of Wnt signaling blocks the compound effects on differentiation

Conclusions:

- PPAR-sparing differentiation of BAT and mitochondrial biogenesis appear to be due to attenuation of Wnt signaling pathways and modulation of nutrient-sensing pathways independent of direct effects on PPARγ
- These effects of PPAR-sparing MSDC-0160 might explain the lack of weight gain in diabetic subjects in clinical trials
- Compounds affecting these pathways independent of direct activation of PPAR transcription factors may pave the way for a new approach to treat type 2 diabetes
- Interestingly, mutations in Wnt signaling are associated with diabetes susceptibility (e.g., Nat Genet 2006; 38:320; Am J Hum Genet 2004; 75:832; Nutr Metab Cardiovas Dis 2009; 19:140)

See also 0092-OR, 1728P, 1729P, 1973P