New Insulin Sensitizers Produce Differentiation of Brown-like Adipose Cells from a Subcutaneous Fat Depot and Increase Secretion of Adiponectin \textit{in vitro}


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Presenter Disclosure

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Mechanism of Action for Insulin Sensitizers

**Old**
- Troglitazone; Rosiglitazone; Pioglitazone

**New**
- MSDC

**Original TZDs**
- PPAR\(\gamma\)
  - Cell Nucleus
  - PPAR-Driven Gene Changes
  - Fat Sequestered
  - Increased Insulin Action
  - Fluid Retention
  - Weight Gain

**MSDC-0160**
**MSDC-0602**
(Phase 2 clinical trials)

**Mito Target of TZDs** (mTOT)
- Metabolic signals
- Nuclear Regulatory Factors
  - Improved Insulin Action
  - Improved Lipid Profiles
  - Regeneration of Brown Fat
  - Regeneration of \(\beta\)-cells
This Presentation:
Compounds also stimulate brown-like phenotype in precursors from axillary fat pads and stimulate production and secretion of adiponectin in a PPARγ-independent manner.
TZDs Increase Differentiation of Brown Fat Progenitors

- The effect of TZDs on BAT is maintained in new insulin sensitizers.
- Similar to pio and rosi (although > 30-fold, 10-fold reduction at PPARγ vs rosi, pio).
- Not blocked by PPARγ antagonists; signaling occurs in PPARγ-KO cells.

Will new insulin sensitizers affect subcutaneous fat?
> Adiponectin production/secretion?
Methods

- Progenitor cells are isolated from axillary fat pads from 3-4 week old CD-1 mice and cultured for 7 days in DMEM + 10% FBS.

- At 90% confluence the cells are treated with various concentrations of compounds (172 nM insulin); medium is changed every 48 hours with fresh additions.

- Cells are harvested for mRNA analysis (rt-PCR) and Western Blots at various time points.

- Conditioned medium is harvested for measurement of secreted adiponectin by ELISA.

• Pad isolated from 10-15 mice
• Digested with collagenase
• Precursors isolated and plated
• Grown to 90% confluence
Conversion of Progenitor Cells to Brown-like Phenotype (7 days of treatment)

- Multilocular fat droplets
- Increased Mitochondria
- Increased UCP1 (message and protein)
- Increased adiponectin (message, protein, and secretion)
PPARγ Antagonists Do Not Block Compound-Induced Effects on UCP1

Note: antagonists affect baseline UCP1 mRNA and differentiation in absence of compounds and rosi action in a PPARγ cell assay but do not block the effects of the compounds to increase UCP1 mRNA and protein.
Increase in UCP1 Protein Expression in Subcutaneous Adipose Progenitors

New Insulin Sensitizers Increase UCP1 Message and Protein In SC Adipose

Western blot UCP1- 6 days

Time (days)

Dose (μM)

New Insulin Sensitizers Increase UCP1 Message and Protein In SC Adipose
New Insulin sensitizers also increase mitochondrial biogenesis by a mechanism independent of PPARγ activation in SC adipose progenitors.
New insulin sensitizers directly increase adiponectin production in subcutaneous adipose tissue independent of expansion of white fat or activation of PPARγ.
Summary

• New insulin sensitizing agents cause browning of progenitor cells from the axillary fat pad in a PPAR-independent manner.
  – Not related to ability to bind to and activate PPARγ - rosi vs 0160 and 0602
  – Not blocked by PPARγ antagonists

• This mechanism includes increase in UCP1 and mitochondrial biogenesis.
  – mRNA
  – Protein

• The compounds increase adiponection in a PPARγ-independent manner.
  – Expression
  – Secretion into the medium

- New insulin sensitizers not only stimulate differentiation of dedicated brown fat progenitor cells but also favor brown adipose-like phenotype and increase adiponectin secretion from subcutaneous adipose.

- There is potential for a new generation of insulin sensitizing agents that avoids side effects associated with activation of PPARγ.
Implications for Novel Agents

New Insulin Sensitizers

- mTOT
- PPARγ

- BAT
- Liver
- Muscle
- Other Tissues

- Nutrient Signaling
- White Adipose
  - "Browning" Favoring Lipid Oxidation Over Storage

- Adiponectin

- Insulin Sensitivity
  - Without PPAR-liabilities

First generation Insulin Sensitizers

- mTOT
- PPARγ

- >White Adipose
  - Adiponectin

- Brain-food intake

- Insulin Sensitivity
  - With Fat Sequestration,
  - Weight Gain, Fluid Retention