ENHANCEMENT OF BROWN ADIPOSE TISSUE DEVELOPMENT \textit{IN VIVO} BY A NOVEL INSULIN SENSITIZER

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Presenter Disclosure

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Board Member/Cofounder: Metabolic Solutions Development Co., LLC
Employee: Metabolic Solutions Development Co., LLC
Stock/Shareholder: Metabolic Solutions Development Co., LLC
Mechanism of Action for Insulin Sensitizers

Old
Troglitazone; Rosiglitazone; Pioglitazone

Original TZDs

PPAR\(\gamma\)
Cell Nucleus

PPAR-Driven Gene Changes

Fat sequestered
Increased Insulin Action
Fluid Retention
Weight Gain

New
MSDC

Mito Target of TZDs (mTOT)

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

Metabolic signals

Nuclear Regulatory Factors

Improved Insulin Action
Improved Lipid Profiles
Increased Brown Fat
Preservation of \(\beta\)-cells
PPARγ Sparing Clinical Candidates

- Pioglitazone is less PPARγ activating than rosiglitazone
- Compounds can be identified that are significantly less PPAR activating
- PPAR-sparing compounds are able to increase brown adipose tissue in a PPAR-independent manner.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (µM)</th>
<th>EC50- pio1</th>
<th>Fold pio2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosiglitazone</td>
<td>0.148</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>1.623</td>
<td>9.57</td>
<td>12.96</td>
</tr>
<tr>
<td>MSDC-0160</td>
<td>23.73</td>
<td>14.62</td>
<td>9.58</td>
</tr>
<tr>
<td>MSDC-0602</td>
<td>15.54</td>
<td>27.86</td>
<td>26.58</td>
</tr>
<tr>
<td>MSDC-0597</td>
<td>6.15</td>
<td>3.79</td>
<td>3.50</td>
</tr>
</tbody>
</table>

1 Lantha screen –binding
2 Gene blazer- cell activation
mTOT Mitochondrial Target of TZDS
Compounds that compete increase UCP1 expression

Photoaffinity crosslinking

1. DMSO
2. MSDC-0160
3. MSDC-0602
4. Pioglitazone

UCP1 Western Blot

- MSDC-0160
- Pioglitazone
- Rosiglitazone

MSDC-0160 (µM)

0 0.1 0.3 1 3 10

0 1000 2000 3000 4000 5000 6000

UCP (density Western blot)

~32kDa
Increase in UCP1 Protein is Not Blocked By PPARγ Antagonists — BAT and Axillary Progenitors

Pre-treatment with or without compounds and antagonist for 6 days

Source of progenitors
Intrascapular BAT

Axillary adipose

<table>
<thead>
<tr>
<th>Source of progenitors</th>
<th>T0070907 (1 μM)</th>
<th>Rosi (0.3 μM)</th>
<th>MSDC-0160 (1 μM)</th>
<th>GW9662 (1 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrascapular BAT</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Axillary adipose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

See also poster 1603P Bill McDonald, et al. Novel Insulin Sensitizers Enhance Brown Adipose Cell Differentiation by Modulation of the Wnt Signaling Pathway
Time Course of Effects *In Vitro*

mTOR activity is measurably reduced after 2 days of treatment.

**OxPhos Western**

MSDC-0160 µM 6 days of treatment

2-ddCt (Treatment/Control)

Days of Treatment

Note: mitochondrial biogenesis with little effect on PGC1α

Note different scale for UCP1

- CPT1b
- UCP1
- PPARα
- PPARγ
- FABP4
- PGC-1α
Evaluate MSDC-0160 in PGC1α Null Mice

PGC1α null mice on C57BL/6 background [Burgess, et al. *J. Biol. Chem.*, 2006; 281: 19000 – 19008]

- Effects on isolated progenitor cells *in vitro* WT and KO
  - Differentiation
  - UCP1 expression
  - Mitochondrial biogenesis

- Treatment of Wild Type and PGC1α knockout mice *in vivo* for 30 days with 30 mg/kg MSDC-0160. Tissues harvested and evaluated.
  - Intrascapular brown fat
  - Perirenal fat
  - Epididymal fat
The effects of MSDC-0160 are independent of PGC-1α, Major PPARγ Coactivator (BAT precursors)

- The effects of MSDC-0160 are independent of PGC-1α
- BAT phenotype, mitochondrial biogenesis, FFA oxidation are favored
• MSDC-0160 increases functional brown fat in WT and PGC-1α KO mice
• In contrast epididymal fat (white adipose tissue) is reduced in mass
• Body weight tends to be reduced

WT = wild type
KO = PGC-1α knock out
Epididymal Adipose Pad treatment of C57 mice in vivo

30 mg/kg for 30 days

- Unlike BAT, MSDC-0160 decreased the epi fat pad in WT (not KO) mice
- Increase in UCP1 and CPT1b in both WT and KO
- Dissection of gastrocnemius indicated that like BAT it was increased in size.

WT = wild type
KO = PGC-1α knock out
Increase in Perirenal UCP1 Expression in WT Mice (mixed response in PGC1α KO mice)

Control

Browning in perirenal adipose

MSDC-0160

Response blocked in PGC1α KO
PPAR$_{\gamma}$-sparing TZDs Cause Adipocyte ‘Browning’ in ob/ob Mice As Well

Ob/ob mice treated for 4 weeks - Epididymal fat pad

While some drug effects may not be dependent on PGC1$_{\alpha}$, overall effects include these pathways

*See also poster 1728P* Brian Finck, *et al.* Insulin Resistance in ob/ob Mice Is Ameliorated by Thiazolidinediones That Do Not Activate PPAR$_{\gamma}$
Effects Independent of PPARγ

Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle

- Similar number of progenitor cells from WT and KO pads
- Full differentiation is arrested in these mice
- However, responses to MSDC-0160 are the same in progenitor cells from WT and KO
Some Effects Persist in PPARγ-null Cells

Increased Mitochondrial Biogenesis
Western Blot

Increased UCP1 message
Knockout

NOTE:
• Differentiation of BAT cells is attenuated in the PPARγ Knockout
• Increase in UCP1 message is 100 fold reduced and protein does not increase
• However, drug induced actions still occur similarly in both the WT and KO
Effects in PPARγ-null Cells
(compounds at 1 μM for 6 days) TZD and non-TZD compounds

Knockout

Wild Type

- MSDC-0160 and MRL-24 have similar effects in the knockout as in the wild type.
- Both message and protein are increased independent of PPARγ or differentiation.

C= DMSO
M= 0160

aP2 Western

Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARgamma by Cdk5.
JH Choi, AS Banks, JL Estall, S Kajimura, P Bostrom, D Laznik, JL Ruas, MJ Chalmers, TM Kamenecka, M Bluher, PR Griffin, and BM Spiegelman
Effects in PPARγ-null Cells
(compounds at 1 μM for 6 days) TZD and non-TZD compounds

Adiponectin

Knockout

- MSDC-0160 and MRL-24 have similar effects in the knockout as in the wild type.
- Drug-induced increase independent of PPARγ.

Wild Type
Mechanism of Action for Insulin Sensitizers

**Old**  
Troglitazone; Rosiglitazone; Pioglitazone  
Original TZDs

- **PPARβ**  
- **Cell Nucleus**

PPAR-Driven Gene Changes

- Fat Sequestered
- Increased Insulin Action
- Fluid Retention
- Weight Gain

**New**  
MSDC

- **Mito Target of TZDs (mTOT)**

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

- Nutrient signaling (Wnt pathway)

- **Cell Nucleus**

- **Nuclear Regulatory Factors**

- **Improved Insulin Action**
- **Improved Lipid Profiles**

- **Increased Brown Fat**
- **Preservation of β-cells**
Conclusions

- Presentations from this Symposium demonstrate the potential for treating diabetes by modifications in adipose or brown adipose tissues.

- These results indicate that a selective mitochondrial action of small molecules currently in clinical trials can augment brown adipose tissue in the intrascapular pad and cause "browning" in other adipose stores.

- The potential benefit of this mechanism is being evaluated in clinical trials.
Extra Slides Regarding Mechanism

*See also poster 1603P* Bill McDonald, *et al*. Novel Insulin Sensitizers Enhance Brown Adipose Cell Differentiation by Modulation of the Wnt Signaling Pathway
Summary of Current Knowledge

- Not blocked by PPARγ antagonists
- Occurs in PGC1α and PPARγ knockouts
- Involves a change in nutrient sensing pathways
- Earliest effect on phosphatase activity
- *Involves modification of Wnt signaling pathway*

*Importance of mTOT in this signaling is currently under intense investigation*

1729-P  White, et al. A Mitochondrial Target of Pioglitazone Acutely Regulates Mitochondrial Respiratory Function
Mitochondrial Molecular Switch for Terminal Differentiation of BAT: Attenuation of Wnt Signaling by the PPARγ-sparing TZDs

- Wnt ligands
- B-catenin
- B-catenin-P
- ubiquitination
- destruction
- GSK3β
- PP2A
- mTOT
- Li, Chi99021
- Pre-programmed changes in gene expression
- Terminal BAT Differentiation
- UCP1
- FFA oxidation program
- Mitochondrial biogenesis

Other transcription factor input
- PPARs, CEBP, FOXO, etc

MSDC-0160
MSDC-0602

Exit cell cycle

BAT progenitor cells


*CONCLUSION: Variation in WNT5B predisposes to T2D in the absence of obesity. The increase in risk conferred by the presence of both WNT5B and TCF7L2 variants strengthens the role of Wnt signaling in T2D.
Pioglitazone and Its Major Metabolites; Other Compounds

Rosiglitazone

Pioglitazone

MSDC-0160

MSDC-0602