Hepatic Effects of NS-0200 (Leucine-Metformin-Sildenafil) in an Obese Model of Diet-Induced and Biopsy-Confirmed NASH

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INTRODUCTION

The Sirt1-AMPK pathway is a key regulator of mitochondrial biogenesis, energy and lipid metabolism, and inflammation; activation of this pathway is a therapeutic target for NASH. NS-0200, a combination of leucine (Leu) with low doses of metformin (Met) and sildenafil (Sil) that synergizes to activate Sirt1-AMPK signalling, has been shown to act against NASH and fibrosis in preclinical models, and is currently in clinical investigation for treatment of NASH.

AIMS

• Compare the histopathological effects of the triplet combination in NS-0200 with two related doublet combinations (Leu-Met and Leu-Sil) in a validated rodent model (Gubra DIO-NASH Mouse)

• Determine the effects of NS-0200 with Leu-Met and Leu-Sil on relevant hepatic gene regulatory pathways in the Gubra DIO-NASH mouse.

METHODS

Figure 1. Study Design

RESULTS

Table 1. Study Groups (All Doses P.O. b.i.d.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Treatment</th>
<th>BMI</th>
<th>Gender</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOW</td>
<td>Control</td>
<td>CHOW Vehicle (BID, PO)</td>
<td>CHOW</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO</td>
<td>Control</td>
<td>CHOW Vehicle (BID, PO)</td>
<td>DIO</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Metformin</td>
<td>LeuMet, (BID, PO)</td>
<td>DIO-NASH</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Sildenafil</td>
<td>LeuSil (1.25), (BID, PO)</td>
<td>DIO-NASH</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Metformin + Sildenafil</td>
<td>LeuMetSil (1.25), (BID, PO)</td>
<td>DIO-NASH</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Leucine</td>
<td>Leu, (BID, PO)</td>
<td>DIO-NASH</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Leucine + Metformin</td>
<td>LeuMet, (BID, PO)</td>
<td>DIO-NASH</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Leucine + Sildenafil</td>
<td>LeuSil (1.25), (BID, PO)</td>
<td>DIO-NASH</td>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>DIO-NASH</td>
<td>Leucine + Metformin + Sildenafil</td>
<td>LeuMetSil (1.25), (BID, PO)</td>
<td>DIO-NASH</td>
<td>Male</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 2. Fibrosis scoring (left panel). For each group, the number of animals with changes in fibrosis (shown in box) or, among animals with changes in fibrosis, the change in fibrosis score (shown in line) to depict the height of the stacked bar. Lower graphs of individual animal data organized by treatment group.

Figure 3. Liver Col1a1 (IHC) (post) expression level (RPKM) that synergizes to activate Sirt1 in the NASH vehicle control group. A colored point for the gene and treatment indicates if the fold change is significantly different from the NASH vehicle. Bold values represent the day of treatment, with treatment times indicated in the legend. Fold changes for all genes in the stellate cell activation pathway vs. the NASH vehicle control group. A colored point for the gene and treatment indicates if the fold change is significantly different from the NASH vehicle. Bold values represent the day of treatment, with treatment times indicated in the legend.

Figure 4. Plot changes for all genes in the stellate cell activation pathway vs. the NASH vehicle control group. A colored point for the gene and treatment indicates if the fold change is significantly different from the NASH vehicle. Bold values represent the day of treatment, with treatment times indicated in the legend.

Figure 5. Plot changes for all genes in the stellate cell activation pathway vs. the NASH vehicle control group. A colored point for the gene and treatment indicates if the fold change is significantly different from the NASH vehicle. Bold values represent the day of treatment, with treatment times indicated in the legend.

CONCLUSIONS

Although NS-0200, Leu-Met, Leu-Sil and Leu-Met-Sil all significantly regress steatosis, inflammation and ballooning in this model, only NS-0200 exerted a robust effect on fibrosis and Col1a1, as well as on stellate cell activation, monocyte recruitment, and FXR targets.

REFERENCES

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• Vapalic RA, Siddiqui M, Rinello CA, Kosinski M, Flores O, Zemel MB, Chasapis N. Effects of NS-0200 at three non-alcoholic steatohepatitis combination, on non-alcoholic fatty liver disease. Diabetes 2017; 66 (suppl1A):17-L4B (abstract)

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