A novel semicarbazide-sensitive amine oxidase inhibitor, TERN-201, reduces NAS and fibrosis in rodent models of non-alcoholic steatohepatitis

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INTRODUCTION

Semia (Semia-carbazide-sensitive Amine Oxidase, VAP-1, AOC3) is a dual function cell cell membrane molecule with amine oxidase activity. It is also involved in the overall immune response to injury.  Semia knock out mice are protected from steatohepatitis.  Semia is also involved in the modulation of the immune response during the repair phase.

A novel mechanism for cytokines to be involved in the development of fibrosis is that cytokines stimulate MAO activity, and MAO products may be involved in the development of fibrosis.

AIM

To evaluate the efficacy and mechanism of action of TERN-201 in two rodent models of liver disease.

METHOD

- IC50 values of SSAO, MAO-A, MAO-B and DAD inhibition were monitored by following the oxidation of the luminogenic amine substrate in the MAO-Glo™ assay kit (Promega).  TERN-201 was pre-incubated with recombinant proteins for 10min at RT before the addition of substrate (10 μM).  The oxidation of the substrate was conducted for 2h after the addition of the detecting reagents.  The luminescence intensity recorded was converted to percent inhibition using values obtained from the MN and MAX controls run on the same plate.  The rate of SSAO inhibition was measured using benzylamine as the substrate and following H2O2 production using the H2O2 Perspex Red kit (Molecular Probes).  The reaction was initiated by the addition of SSAO at RT.  The fluorescence intensity was monitored for 1h.  The intensity change over time was fitted to a first order process to calculate the rate of inhibition.  The inhibition rate constant (k10%) was determined from the initial linear slope of the dose response.
- SD rats were dosed PO with vehicle or TERN-201 (n=6/group).  Liver samples were collected at 2 or 24h post dosing. SSAO activity was determined by measuring the total amine and oxidase activity in the presence of the SSAO inhibition.  Cytochrome P450, and Pargyline, using the MAO-Glo™ assay kit. Tissue lysates were prepared by homogenization in lysis buffer and incubated with Cytochrome (10 μM) and Pargyline (10 μM) at RT before detecting the luciferase activity in the presence of 10 μM DOPA, 10 μM Pargyline, 50 μl P4A4226 and 10 μM Hydroxyamine in reaction to be used as "MN" control (0 SSAO activity).  The product generated was measured and quantified after the addition of reagents according to the manufacturer’s protocol. Relative SSAO activity % (of control) was calculated as (sample/g of MN controls)/mean of Vehicle/g of MN control.
- C57BL mice fed a high-fat diet (D12492, Research Diet), fat/protein/carbohydrate 60/20/20 Kcal%, 10w) or non-obese SD rats were dosed PO with TERN-201 daily and biweekly IP or intraperitoneal PO carbon tetrafluoride (CCl4) treatment, respectively.

RESULTS

Table 1. TERN-201 is a potent and selective irreversible SSAO inhibitor

<table>
<thead>
<tr>
<th>Enzyme Inhibition</th>
<th>TERN-201</th>
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<tbody>
<tr>
<td>SSAO</td>
<td>IC50 10.8/23.3/12.5 (h/r/m; nM)</td>
</tr>
<tr>
<td>SSAO (inactivation rate constant) (IC50/IC50)</td>
<td>19706/24655/19556 (h/r/m; M^2 s^-1)</td>
</tr>
<tr>
<td>MAO-A</td>
<td>IC50 &gt;50 (h/μM)</td>
</tr>
<tr>
<td>MAO-B</td>
<td>IC50 &gt;200 (h/μM)</td>
</tr>
<tr>
<td>DAD</td>
<td>IC50 &gt;936 (h/μM)</td>
</tr>
</tbody>
</table>

h-human/rat/mouse

Figure 1. TERN-201 inhibits rat liver SSAO activity >90% for 24h with a single dose

Figure 2. TERN-201 reduces liver inflammation, fibrosis and immune and fibrosis-associated genes in a rat CCl4 fibrosis model

Figure 3. TERN-201 reduces NAFLD Activity Score (NAS) in HFD/CCl4 NASH mice

Figure 4. TERN-201 causes a dose-dependent reduction in an immune system RNA signature in HFD/CCl4 NASH mice

REFERENCES


CONCLUSIONS

- TERN-201 is a potent and selective irreversible SSAO inhibitor in vitro and in vivo.
- TERN-201 suppressed liver fibrosis and inflammation in a rat model of liver disease and caused a dose-dependent reduction in NAFLD activity score in a mouse NASH model.
- TERN-201 reduced an immune system RNA signature in the mouse model of NASH in a dose-dependent manner.

DISCLOSURES

Authors are employees/stockholders in Terns Pharmaceuticals

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