**BACKGROUND AND AIM**

Apoptosis Signal-Regulating Kinase 1 (ASK1) is a redox-sensitive kinase. In the setting of oxidative stress, ASK1 activates mitogen-activated protein kinase (MAPK) signalling which, in turn, modulates the activity of apoptotic and inflammatory pathways. As a result, inhibition of ASK1 has been proposed as a therapeutic approach for the treatment of non-alcoholic steatohepatitis (NASH). EP-027315 is a novel, highly selective, and potent (IC₅₀ < 1.25 nM) ASK1 inhibitor. Here, we evaluate the in vivo efficacy of EP-027315 in mouse models of liver injury (cytotoxicity and metabolic disease - diet-induced obesity).

**RESULTS**

**EP-027315 is a Potent and Selective ASK1 Inhibitor**

<table>
<thead>
<tr>
<th>ASK1 Inhibitor Assays</th>
<th>EP-027315</th>
<th>GS-4997</th>
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</thead>
<tbody>
<tr>
<td>Biochemical IC₅₀ (nM)</td>
<td>&lt;1.25</td>
<td>&lt;1.25</td>
</tr>
<tr>
<td>Cell-Based IC₅₀ (nM)</td>
<td>3.4±1.1</td>
<td>2.8±1.1</td>
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<tr>
<td>Binding Affinity Kᵟ (nM)</td>
<td>0.26±0.06</td>
<td>0.3±0.1</td>
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<tr>
<td>Selectivity Score</td>
<td>1.3%</td>
<td>7%</td>
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</table>

**In Vivo.** Biochemical IC₅₀ of ASK1 inhibitors (AS11K) was determined by TR-FRET (Cisbio KinEASE STK 5.3 kit). Cell-based IC₅₀ was evaluated in hydrogen peroxide (H₂O₂)-treated HEK293 cells stably expressing human ASK1, pre-incubated with ASK1. Binding affinity (Kᵟ) and kinase selectivity (ScanMax) assays were performed by DiscoverX.

**In Vivo.** Inhibition of ASK1 activation was tested in C57BL/6 male mice (8 weeks of age) receiving a single dose of vehicle or EP-027315 (oral gavage) 1 hour prior to acetaminophen (APAP) administration (300 mg/kg). Livers and plasma were harvested 6 hours after APAP treatment to evaluate the effects of EP-027315 on hepatic ASK1 signalling. To further characterize the pharmacologic effects of ASK1 inhibition on APAP-mediated hepatotoxicity, RNA-Seq analysis of livers was performed. In addition, inhibition of ASK1 was evaluated in diet-induced obese (DIO) mice fed a high-fat diet (D12492) for 24 weeks and dosed with ASK1 for 2 consecutive days. Three hours after the last dose, liver was harvested for protein and RNA analysis. In both models, MAPK signalling and apoptotic markers were evaluated using immunoblot or MSD (Mesoscale DiscoverX) assays. Hepatic injury and inflammation were assessed by plasma ALT, ALP, γ-GT, and histological evaluation of formalin-fixed tissues.

**RESULTS**

**EP-027315 Protects Mice from Acute Liver Injury**

**EP-027315 Protects Mice from Diet-Induced Liver Injury**

**CONCLUSION**

- EP-027315 is a potent and selective inhibitor of ASK1.
- EP-027315 inhibits hepatic ASK1 and blocks liver injury induced by either acute toxicity or chronic metabolic stress.
- EP-027315 treatment effectively suppressed markers of liver injury, inflammation, and apoptotic pathways at both a transcriptional and protein level.
- These data support further evaluation of EP-027315 for the treatment of NASH.

**ACKNOWLEDGEMENTS**

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