In vivo effects of a novel inhibitor of apoptosis signal-regulating kinase 1 (ASK1) in mouse models of liver injury and metabolic disease

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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 (IC_{50} < 1.25 nM) ASK1 inhibitor. Here, we evaluate the *in vivo* efficacy of EP-027315 in mouse models of liver injury (acetaminophen toxicity) and metabolic disease (diet-induced obesity).

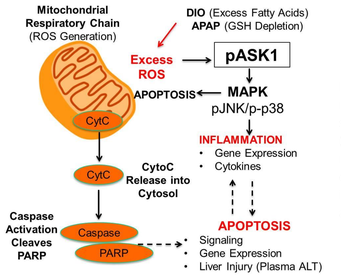

Figure1. ASK1 activation in response to excess reactive oxygen species (ROS) due to glutathione (GSH) depletion in liver injury model of acetaminophen (APAP), or due to excess fatty acids in diet-induced obesity (DIO), leads to apoptosis and inflammation.

METHODS

In Vitro. Biochemical IC_{50} of ASK1 inhibitors (ASK1i) was determined by TR-FRET (Cisbio KinEASE-STK S3 kit). Cell-based IC_{50} was evaluated in hydrogen peroxide (H_2O_2)-treated HEK293 cells stably expressing human ASK1, pre-incubated with ASK1i. Binding affinity (KdElect) 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, i.p). 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 ASK1i 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 Discovery) assay. Hepatic injury and inflammation were assessed by plasma ALT, plasma IL-1β, and histological evaluation of formalin-fixed tissues.

RESULTS

EP-027315 Is a Potent and Selective ASK1 Inhibitor

ASK1 Inhibitor Assays	EP-027315	GS-4997
Biochemical IC_{50} (nM) TR-FRET	<1.25	<1.25
Cell-Based IC_{50} (nM) pJNK Inhibition,H_2O_2-treatedHEK293	3.4 ± 1.1	2.8 ± 1.1
Binding Affinity K_d (nM) DiscoverX qPCR-based assay	0.26 ± 0.06	0.3 ± 0.1
Selectivity Score %Kinases Targeted with Kd<100nM DiscoverX ScanMax (468 kinases)	**1.3%**	7%

Table 1. Comparison of the *In vitro* characteristics of EP-027315 and GS-4997 Selonsertib.

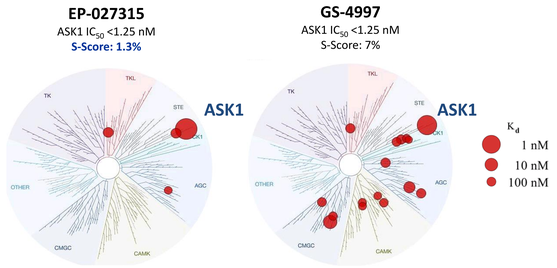

Figure 2. Kinome visualization of off-target kinases with high binding affinity (K_d < 100 nM). Circle size is proportional to binding affinity.

EP-027315 Inhibits MAPK Signaling *In Vivo*

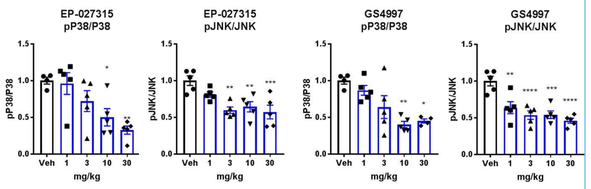

Figure 3. Dose dependent inhibition of liver p-p38 and p-JNK by EP-027315 or GS-4997. Male C57BL/6 male mice were treated for 2 days with the indicated dose of ASK1i by oral gavage. Animals were sacrificed 3 hours after the final dose. Phosphorylation of p38 and JNK normalized to total protein was measured by MSD. One-way ANOVA analysis vs vehicle (Veh). *<p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

RESULTS

EP-027315 Protects Mice from Acute Liver Injury

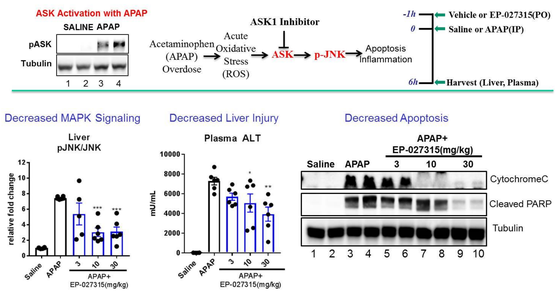

Figure 4. EP-027315 decreases liver pJNK, plasma ALT, and apoptosis in a dose-dependent manner. One-way ANOVA analysis vs APAP (n=6). *<p<0.05, **p<0.01, ***p<0.001.

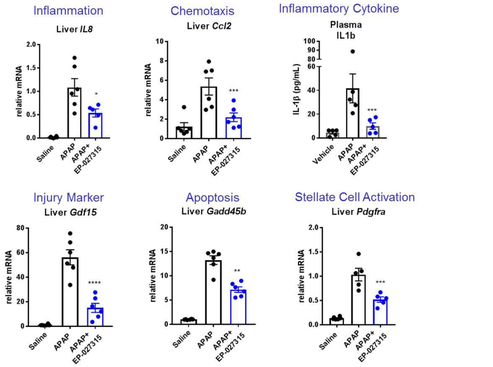

Figure 5. qPCR validation of liver RNAseq. EP-027315 (30 mg/kg) blunted inflammatory, apoptotic, and liver damage-associated genes increased by APAP. Plasma IL1β was also decreased. One-way ANOVA analysis vs APAP (n=6) *<p<0.05, **p<0.01, ***p<0.001, ****p<.0001.

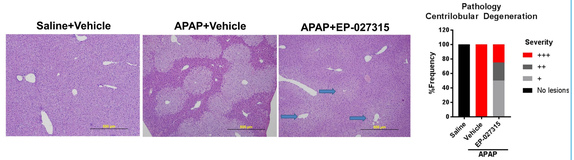

Figure 6. Histological evaluation (H&E of liver damage by APAP). Blue arrows indicate regions with decreased centrilobular hepatocyte degeneration due to EP-027315 (30 mg/kg).

RESULTS

EP-027315 Protects Mice from Diet-Induced Liver Injury

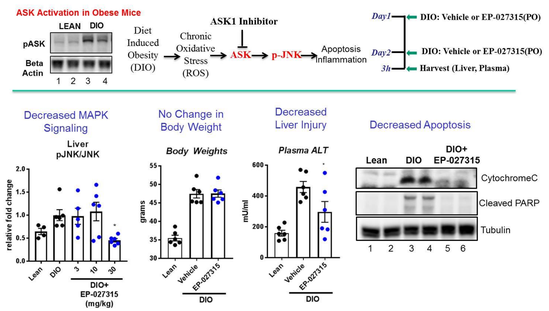

Figure 7. EP-027315 (30 mg/kg) decreases liver pJNK, plasma ALT, and apoptotic signaling in DIO mice without changing body weight. One-way ANOVA analysis vs DIO (n=6) *<p<0.05.

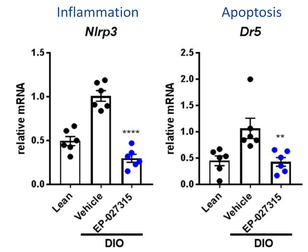

Figure 8. qPCR of differentially expressed hepatic genes in DIO mice, which are decreased by the ASK1 inhibitor EP-027315 (30 mg/kg). One-way ANOVA analysis vs APAP (n=6). **p<0.01, ****p<0.0001.

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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