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

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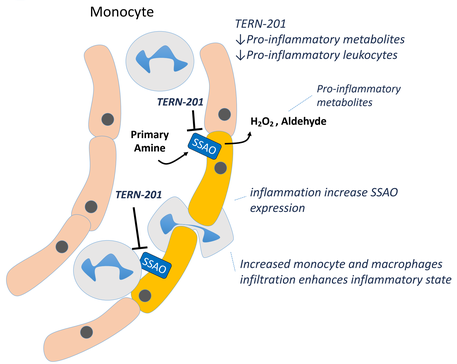
1 INTRODUCTION



SSAO (Semicarbazide-Sensitive Amine Oxidase, VAP-1, AOC3) is a dual function cell adhesion molecule with amine oxidase ecto-enzyme activity that catalyzes the oxidative deamination of primary amines to aldehyde and hydrogen peroxide (H_2O_2) increasing systemic oxidative stress and damage to vasculature. SSAO is upregulated in endothelial cells at sites of inflammation enhancing leukocyte entry into inflammatory sites.

Serum SSAO is significantly increased in patients with histologically confirmed NASH and independently associated with fibrosis stage (Weston et al.). In rodent models, SSAO deficiency attenuates CCl_4-induced hepatic fibrosis and SSAO knockout mouse liver exhibits reduced steatosis and fibrosis when fed with a western lifestyle diet (Weston et al.).

Pharmacological SSAO inhibition is anticipated to reduce inflammation and fibrosis in NASH patients. This novel mechanism provides an appealing opportunity to treat NASH, a disease with significant unmet medical needs. TERN-201, a novel, selective SSAO inhibitor, has entered Phase 1 clinical trials.

2 AIM

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

3 METHOD

- IC_{50} values of SSAO, MAO-A, MAO-B and DAO 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 oxidase proteins for 10min at RT before the addition of substrate (10 µM). The oxidation of the substrate was conducted for 2h before the addition of the detecting reagents. The luminescence intensity recorded was converted to percent inhibition using values obtained from the MIN and MAX controls run on the same plate. The rate of SSAO inhibition was measured using benzylamine as the substrate and following H_2O_2 production using the HRP/Amplex Red kit (Molecular Probe). 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 (kin/Ki) 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 oxidase activity in the presence of the MAO inhibitors, Clogyline, and Pargyline, using the MAO-Glo™ assay kit. Tissue lysates were prepared by homogenization in lysis buffer and incubated with Clogyline (10 µM) and Pargyline (10 µM) at RT before adding the luminogenic substrate for 1h at 37ºC. Tissue lysate from vehicle group was also incubated with 10 µM Clogyline, 10 µM Pargyline, 50 µL PXS4728A, and 10 µM Hydroxylamine before reaction to be used as "MIN" control (0 SSAO activity). The product generated was measured and quantified after the addition of detecting reagent according to the manufacture's protocol. Relative SSAO activity (% of control) was calculated as (sample – mean of MIN control)/(mean of Vehicle –mean of MIN control).
- C57BL6 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 triweekly PO carbon tetrachloride (CCl_4) treatment, respectively.

4 RESULTS

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

Enzyme Inhibition	TERN-201
SSAO	IC_{50} =10.8/23.3/12.5 (h/r/m; nM)
SSAO Inactivation rate constant (k_{in}/K_i)	19706/24655/19556 (h/r/m; $M^{-1}S^{-1}$)
MAO-A	IC_{50} >50 (h; µM)
MAO-B	IC_{50} >200 (h; µM)
DAO	IC_{50} =936 (h; nM)

h-human/r-rat/m-mouse

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

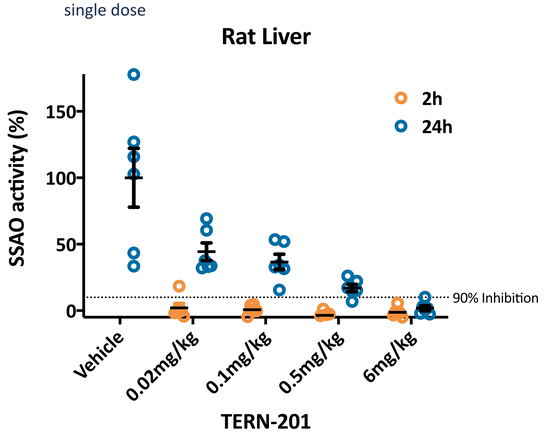
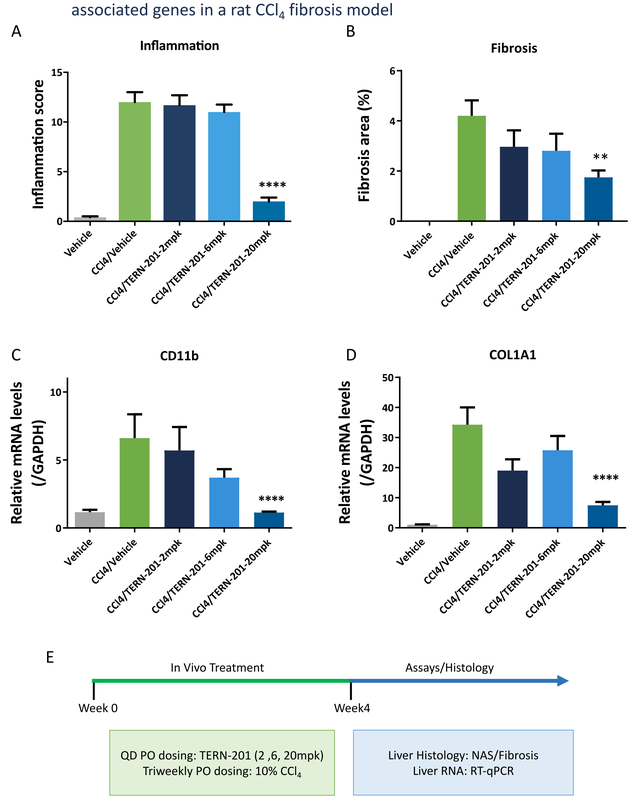
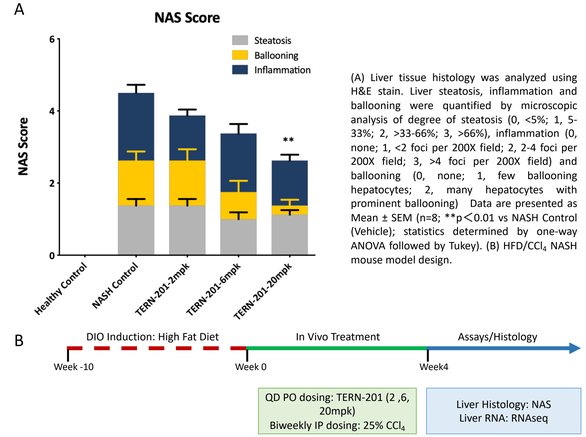


Figure 2. TERN-201 reduces liver inflammation, fibrosis and immune and fibrosis-associated genes in a rat CCl_4 fibrosis model



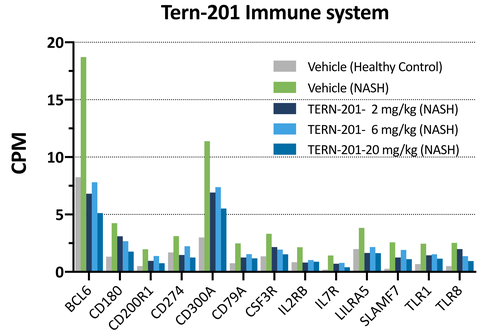
(A) Liver inflammation was quantified by histological analysis of the degree of inflammation (H&E stain, semi-quantitative score of 0-4 with 4 worst). (B) Liver fibrosis was quantified by histological analysis of the percentage of Sirius Red-positive liver sections. (C, D) Liver RNA was analyzed using RT-qPCR with primers for rat CD11B (C) and COL1A1 (D). Data are presented as Mean ± SEM (n=10 for all groups; ****p<0.0001, **p<0.01 vs CCL_4 /Vehicle; statistics determined by one-way ANOVA followed by Dunnett's). (E) Rat CCl_4 model design.

Figure 3. TERN-201 reduces NAFLD Activity Score (NAS) in HFD/CCl_4 NASH mice



(A) Liver tissue histology was analyzed using H&E stain. Liver steatosis, inflammation and ballooning were quantified by microscopic analysis of degree of steatosis (0, <5%; 1, 5-33%; 2, >33-66%; 3, >66%), inflammation (0, none; 1, <2 foci per 200X field; 2, 2-4 foci per 200X field; 3, >4 foci per 200X field) and ballooning (0, none; 1, few ballooning hepatocytes; 2, many hepatocytes with prominent ballooning) Data are presented as Mean ± SEM (n=8; **p<0.01 vs NASH Control (Vehicle); statistics determined by one-way ANOVA followed by Tukey). (B) HFD/CCl_4 NASH mouse model design.

Figure 4. TERN-201 causes a dose-dependent reduction in an immune system RNA signature in HFD/CCl_4 NASH mice



Liver RNA was isolated at termination on day 28 and analyzed by RNAseq. Immune system signature was defined using "Reactome" program.

5 REFERENCES

C. J. Weston et al., Vascular adhesion protein-1 promotes liver inflammation and drives hepatic fibrosis. J Clin Invest. 2015, 125, 501-20.

6 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.

7 DISCLOSURES

Authors are employees/stockholders in Terns Pharmaceuticals

8 CONTACT

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