CTS-1027, a potent MMP inhibitor, Protects Against TNFα- and α-Fas-Induced liver Injury

Patricia C. Contreras and Karen Valentino, Conatus Pharmaceuticals, San Diego, CA 92121

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Abstract

Background: CTS-1027 is an orally bioavailable small molecule that is a potent inhibitor of MMP-2, 3, 8, 9, 12, 13 and 14, but not MMP-1. In the liver, in response to a variety of insults, excessive MMP activity may play a role in destruction of the extracellular matrix, and recruitment of inflammatory cells. CTS-1027 was previously evaluated in an extensive phase 2 clinical trial in which a well tolerated chronically administered dose was identified. Thus, it was of interest to evaluate a MMP inhibitor whose safety profile was known in models of acute liver injury in mice.
Methods: CTS-1027 (0.001-30 mg/kg) was administered PO to male mice (C57Bl/6), 30 minutes prior to treatment with TNFα/D-Galactosamine (D-Gln) or the Fas activating antibody (α-Fas). Six hours later, animals were anesthetized with Nembutal and blood taken by cardiac puncture. Plasma ALT activity was determined using a commercially available kit. For survival studies, mice were observed up to 24 hours post-insult. Pharmacokinetic analysis of CTS-1027 was conducted in treatment-naive mice to determine plasma and liver levels.
Results: CTS-1027 dose-dependently decreased plasma ALT activity in the TNFα model. The average ED50 from 4 studies was 0.26 ± 0.08 mg/kg. Twenty-four hour survival was also increased by CTS-1027 (10 mg/kg). The average 24 hour survival from 3 studies was 27 ± 7.3% and 55 ± 7.6% (p=0.03) in the TNFα/D-Gln control mice and CTS-1027-treated mice, respectively. CTS-1027 (10 mg/kg; PO) was also protective against α-Fas, significantly (p<0.05) reducing the elevation in plasma ALT activity in 2 independent studies by an average of 49%. In a separate group of mice, PK analysis of CTS-1027 in plasma vs. liver indicated that CTS-1027 was rapidly absorbed with Tmax of 0.25-0.5 hour in liver and plasma. AUCs increased in a dose-dependent manner and were 1.7-fold greater in liver than plasma. The AUC in plasma at the ED50 dose in the TNFα/D-Gln model (~0.3 mg/kg) was 13-fold lower than the AUC at a dose previously determined to be well tolerated in man.
Conclusions: CTS-1027 potently antagonized the liver toxicity induced by treatment with TNFα/D-Gln or α-Fas as indicated by reductions in plasma ALT and/or lethality/morbidity at doses lower than the well tolerated dose in man. CTS-1027 is an attractive candidate for further development and is expected to enter clinical trials in patients with liver disease by the end of 2007.

Introduction

CTS-1027 (N-Hydroxy-4- {[4-(4-chlorophenoxy)benzenesulfonyl]methy 1} -2,3,5,6-tetrahydropyran-4-carboxamide) is an oral small molecule that selectively and potently inhibits MMP 2, 3, 8, 9, 12, 13 and 14 at low to subnanomolar KI values. It is a very weak inhibitor of MMP1 and 7. It was originally developed by Roche to treat a different disease. Over 590 subjects (56 normal volunteers and 541 patients) participated in CTS-1027 clinical studies, in which over 500 subjects were exposed to at least one dose of CTS-1027. The incidence of adverse events in CTS-1027 groups employing doses in the range expected for future clinical studies tended to be similar to the placebo group.

Excessive MMP activity that occurs in the liver in response to a variety of insults has been implicated in loss of scaffolding that maintains the normal architecture of the liver and recruitment and activation of inflammatory cells that perpetuate liver damage. Thus, the possibility that CTS-1027 could be protective in models of acute hepatitis was evaluated.

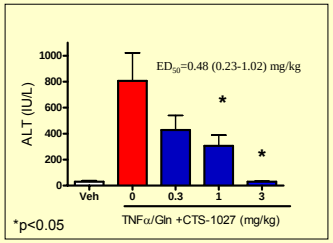
Efficacy Models

Male C57BL/6 mice were used in all studies. The dose and route of administration of TNFa, D(+)galactosamine (GLN), lipopolysaccharide (LPS), activating antibody to Fas (αFas), concanavalin A (Con A) and CTS-1027 are shown in the table below. Blood via cardiac puncture was taken at the times shown in the table, centrifuged and the plasma frozen at -70^0 C until analyzed for ALT activity using a ThermoElectron kit. In some studies, livers were taken at 6 hr for histology.

	Dose and Route for Insult	CTS-1027 (PO) Time of Administration	ALT
TNFα	20-40 μg/kg IP	-30 min	6 hr
αFas	150 μg/kg IV	-30 min	6 hr
LPS/Gln	0.3 μg/kg/700 mg/kg IP	0 or 1.5 hr	8 hr
Con A	25 mg/kg IV	0 hr	16 hr

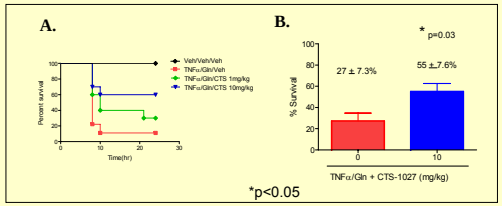
Efficacy in TNFα/Gln Model

Dose-dependent inhibition of ALT



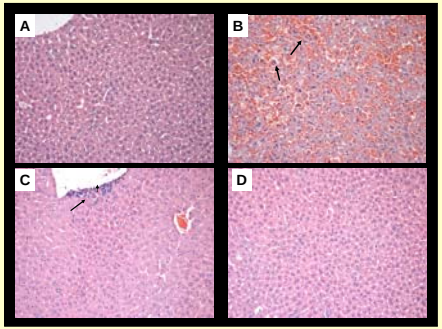
Dose-dependent inhibition of TNFα/Gln-induced Elevation in ALT Activity. CTS-1027 dose-dependently reduced ALT activity. The average ED_{50} from 4 studies was is 0.26 ± 0.08

Improved Survival



CTS-1027 Improved Survival. An example of a survival curve is shown in fig. A. The average from 3 studies (fig. B) shows that CTS-1027 significantly improved 24-hr survival.

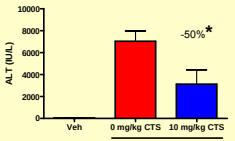
Improved Histology



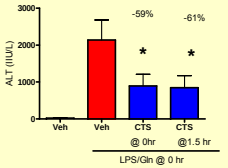
Reduction in hepatocellular apoptosis/necrosis, hemorrhage, and inflammatory infiltrates by CTS-1027. H&E stained murine liver sections 6 hrs after treatment with **A.)** Veh/Veh; **B & C)** Veh (PO) 30 min before TNFα/Gln; and **D)** CTS-1027 (3 mg/kg PO) 30 min before TNFα/Gln. In mice treated with TNFα/Gln and vehicle for CTS-1027, liver injury varied from mild (see C) to severe (see B). In the severe injury (section B), there was hemorrhage, apoptosis (arrows), and necrosis. When the injury was milder, inflammatory infiltration and apoptosis (arrows) was evident (see section C). When mice were pretreated with CTS-1027, hepatic sections were generally unremarkable.

Other Models of Acute Hepatitis

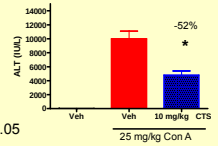
A. Fas



B. LPS/Gln



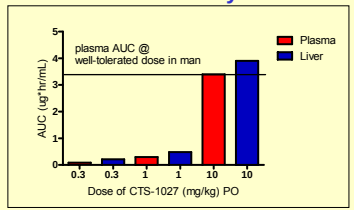
C. Con A



*p<0.05

Examples of hepatoprotection by CTS-1027 in the Fas, LPS/Gln and Con A models. In the Fas LPS/Gln and Con A model, CTS-1027 (10 mg/kg) significantly reduced ALT activity in 2 studies/model by an average of 49%, 51% and 45%, respectively. CTS-1027 reduced ALT activity to the same extent when dosed at the same time or 1.5 hr post administration of LPS/Gln.

PK/PD Relationship : Mouse Efficacy vs. Human Exposure After 28 Days



PK/PK relationship between efficacy in mouse and dose in man. Based on AUC from a 28 day study in man, the ED_{50} dose of CTS-1027 in the TNFα/Gln model is 37-fold lower than a well-tolerated dose in man. Liver AUC averaged 1.7-fold higher than plasma AUC.

Conclusions

CTS-1027 was hepatotoprotective in 4 murine models of acute hepatitis.

- CTS-1027 markedly reduced ALT activity, and improved survival and hepatic histology in the TNFα/Gln model.
- CTS-1027 was as effective when dosed post-insult vs. the same time as insult in the LPS/Gln model.
- CTS-1027 significantly reduced ALT activity in Fas and Con A model where control ALT values were ~7,000-9,000 IU/L.
- AUC values at ED_{50} doses are well below or equivalent to exposure seen in man at doses shown to be safe

These results support the hypothesis that inhibition of MMPs can prevent injury in models of acute hepatitis at doses that should be tolerated in man. Thus CTS-1027 is an attractive candidate for further development and is expected to enter clinical trials in patients with liver disease by the end of 2007.