CRV431 TARGETS THE LIVER AND DECREASES LIVER FIBROSIS IN THE CARBON TETRACHLORIDE MOUSE MODEL

Dr. Daren Ure[1], Dr. Joseph Kuo[2], Mr. Michael Bobardt[2], Mr. Udayan Chatterji[2], Dr. Daniel Trepanier[1], Dr. Philippe Gallay[2] and Dr. Robert Foster[1],

(1) Hepion Pharmaceuticals Inc. (2) Immunology and Microbiology, The Scripps Research Institute

BACKGROUND

The cyclophilin inhibitor, CRV431, was previously shown to decrease levels of liver fibrosis in the murine STAM model of non-alcoholic steatohepatitis (NASH). To determine if CRV431 can decrease fibrosis in another model of liver injury, we investigated CRV431 in the carbon tetrachloride mouse model and compared its activity to obeticholic acid (OCA), a leading late clinical drug candidate for NASH. To define the possible CRV431 mechanisms of action, we also investigated the inhibitory activity of CRV431 towards multiple cyclophilin isoforms implicated in collagen production, cell death, and other pathologic mechanisms.

METHODS

1. CYCLOPHILIN INHIBITION: CRV431 inhibitory potency towards the isomerase activity of cyclophilins A, B, D, and G was measured with a chymotrypsin-coupled peptide assay.

2. CCl_4 MODEL. Liver fibrosis was induced in C57BL/6 mice by intraperitoneal administration of carbon tetrachloride for 6 weeks. Mice were treated by daily oral gavage with CRV431 (50 mg/kg/day), OCA (10 mg/kg/day), or a combination of CRV431 and OCA for the entire 6 weeks. Liver fibrosis was assessed by quantitative morphometry of Sirius Red-stained sections.

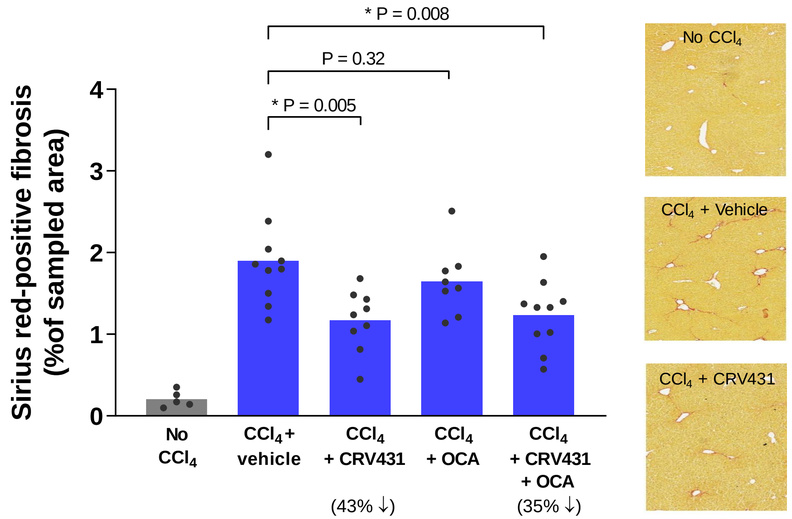
3. CRV431 DISTRIBUTION. CRV431 in blood and liver was quantified by liquid chromatography mass spectrometry.

4. LX2 STELLATE CELLS. CRV431 ± TGFβ were administered to LX2 cells for 2 days. Culture medium from Day 1-2 of treatment was collected, and procollagen and fibronectin concentrations were measured by ELISAs.

RESULTS

Cyclophilin Isoform	CsA IC_{50} (nM)	CRV431 IC_{50} (nM)	IC_{50} fold-difference
PPIA (Cyp A)	25.6 (17.2 nM Ki)	2.5 (1.3 nM Ki)	10.2 (13.2 by Ki)
PPIB (Cyp B)	11.5	3.1	3.7
PPIF (Cyp D)	10.1	2.8	3.6
PPIG (Cyp G)	27.7	7.3	3.8

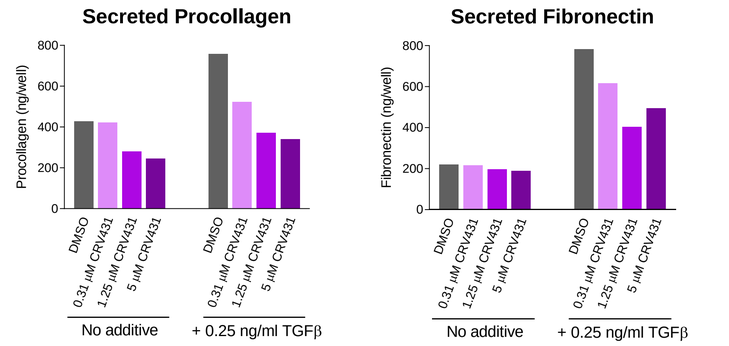
CRV431 inhibits multiple cyclophilin isoforms more potently than cyclosporine A (CsA). Cyclophilin activity was assessed in an *in vitro* enzyme assay based on *cis*-to-*trans* prolyl isomerase of the peptide, N-succinyl-Ala-Ala-Pro-Phe-ρ-nitroanilide.



CRV431 decreases liver fibrosis alone or in combination with OCA in the CCl4 mouse model. 6 weeks of carbon tetrachloride (i.p.) + oral drug treatments. CRV431 was administered at 50 mg/kg/day, and obeticholic acid (OCA) at 10 mg/kg/day. Unpaired t-test analyses.

Species	Disease Model	mg/kg/ day	Day of Dosing	Hours Post-Dose	n	Blood µg/ml	Liver µg/g	Liver:Blood [CRV431] Ratio
Mouse	STAM NASH	50	189	3	10	1.4 ± 1.5	11.6 ± 9.0	**11.9 ± 8.5**
Mouse	CCl_4	50	42	4	9	1.2 ± 0.5	14.5 ± 2.9	**15.4 ± 10.4**
Rat	-	30	7	12	6	1.8 ± 0.3	11.7 ± 1.9	**6.6 ± 1.1**
Rat	-	30	7	24	6	1.3 ± 0.2	6.0 ± 1.4	**4.8 ± 1.1**
Rat	-	250	7	12	6	3.2 ± 0.7	23.4 ± 3.4	**7.6 ± 1.8**
Rat	-	250	7	24	6	2.9 ± 0.6	15.2 ± 4.0	**5.3 ± 0.6**

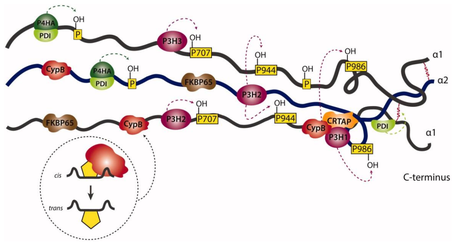
CRV431 blood exposure and liver targeting in mice and rats. CRV431 concentrations were measured in blood and liver from normal rats or diseased mice at the indicated days and times post-dose (once-daily oral gavage). Means ±SD.



CRV431 decreases procollagen and TGFβ-stimulated fibronectin secretion from LX2 cells. LX2 cells in culture were treated as indicated from Day 0-2, and secreted procollagen and fibronectin measured in Day 1-2 culture medium.

CONCLUSIONS

- ✓ These data are consistent with CRV431 directly targeting cyclophilin-mediated, fibrotic processes in stellate cells.
- ✓ The proposed primary anti-fibrotic mechanism is inhibition of cyclophilin B-mediated procollagen synthesis, based on studies demonstrating Cyp B regulation of procollagen hydroxylations and α-helix formation.



Gjaltema and Bank (2017) Molecular insights into prolyl and lysyl hydroxylation of fibrillar collagens in health and disease. Crit Rev Biochem Mol Biol. 52(1):74-95.

DISCLOSURES

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