Cyclophilin inhibitor CRV431 (Rencofilstat) as a potential therapy for ALD

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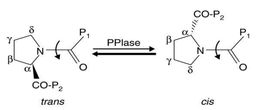
Introduction

Cyclophilins (CyP) are peptidyl-prolyl isomerases that facilitate protein folding and regulate several biological processes with isoforms A, B, D being best characterised. Cyclophilin inactivation via therapeutic inhibition or genetic manipulation has been shown beneficial at various stages of liver disease, including steatosis, fibrosis, inflammation, cell injury and in hepatocellular carcinoma. CRV431 (Rencofilstat) is a pan-cyclophilin inhibitor (non-immunosuppressant cyclosporin derivative (1)) that is currently in clinical development for NASH (Phase 2B).



Functions of CyPs and roles in liver diseases:
- Fibrosis- collagen synthesis/folding including hydroxylation and cross-linking
- Cellular injury- mitochondrial stress, ER stress and cell death
- Steatosis- lipogenesis
- Inflammation- infiltration and activation of inflammatory cells
- Viral Infection- virus entry, replication, etc.
- Cancer- adaptation to hypoxia; metastasis; regulation of cancer cell proliferation

Aims

- To evaluate the therapeutic potential of cyclophilin inhibitor CRV431 (Rencofilstat) in an organotypic experimental model of ALD based on human Precision-cut Liver Slices (PCLS) culture (2)
- To evaluate antifibrotic properties of CRV431 in patient-matched primary human hepatic stellate cells (HSC)

Methods

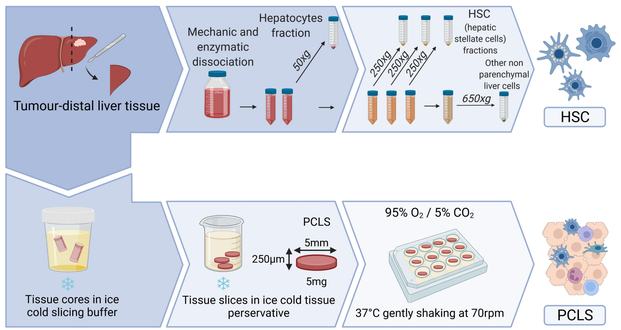


Figure 1: Schematic of the procedures to obtain patient-matched primary HSC culture and PCLS. Patient-matched primary HSCs and Precision Cut Liver Slices (PCLS) were prepared from background (tumour-free) liver specimens derived from patients undergoing secondary liver cancer resection (different fibrotic stages, n=7). Tissue cores were obtained using the Alabama R&D Tissue Coring Press and PCLS sliced with Alabama R&D Tissue Slicer (MD6000).

Table: Baseline characteristics of the liver tissue donors for the production of PCLS and HSC.

	Demographics				Background liver	Tumour		Alcohol	
SUBJECT ID	Gender	Age	Ethnicity	BMI	Fibrosis score	Aetiology	Treatment (Y/N)	current/ former	Units/ week
PCLS-130-KCH	F	81	Caucasian	28.97	F1-F2	CRLM	Y	N	UA
PCLS-132-KCH	M	39	Caucasian	UA	F2-F3	CRLM	N	UA	UA
PCLS-149-KCH	F	37	Caucasian	19.36	F0	CRLM	Y	UA	UA
PCLS-152-KCH	M	40	Caucasian	29.2	F1-F2	CRLM	UA	N	UA
PCLS-156-KCH	F	69	Caucasian	17.3	F0	CRLM	Y	current	<14
PCLS-159-KCH	M	40	Asian	24.8	F1	CRLM	N	N	UA
PCLS-190-KCH	M	60	Caucasian	26.7	F0	CRLM	N	N	UA

Abbreviations: BMI – body mass index, UA – unknown, CLRM – colorectal liver metastasis

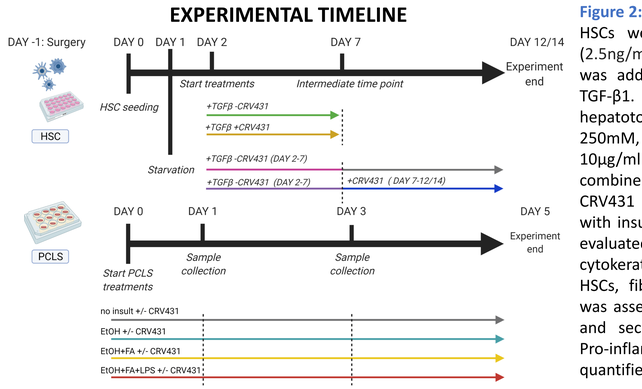


Figure 2: Experimental timeline. HSCs were activated with TGF-β1 (2.5ng/ml) for 5 days. 5µM CRV431 was added simultaneously or after TGF-β1. PCLS were exposed to hepatotoxic insults including ethanol 250mM, fatty acids 0.1mM, LPS 10µg/ml individually and/or combined for up to 5days and 5µM CRV431 was added simultaneously with insults. Tissue functionality was evaluated by histology and cytokeratin-18 release. In PCLS and HSCs, fibrosis/HSC activation status was assessed by gene expression, IF, and secretion of fibrotic markers. Pro-inflammatory cytokines were quantified by Luminex.

Results

CRV431 treatment was not hepatotoxic in PCLS culture

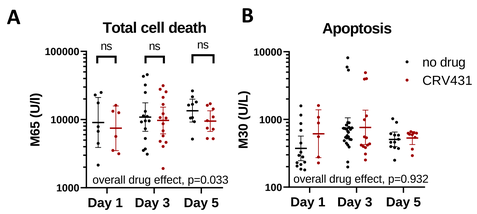


Figure 3: (A) Total hepatocyte death in PCLS measured as a release of cytokeratin-18 (M65 epitope) in culture supernatants. **(B)** Apoptosis in PCLS measured as a release of caspase cleaved epitope (M30) of cytokeratin-18. n≥6, Geomean±95%CI, statistical analysis: 2-way ANOVA adjusted by subject and timepoint.

Addition of CRV431 reduced expression and secretion of profibrogenic markers in PCLS

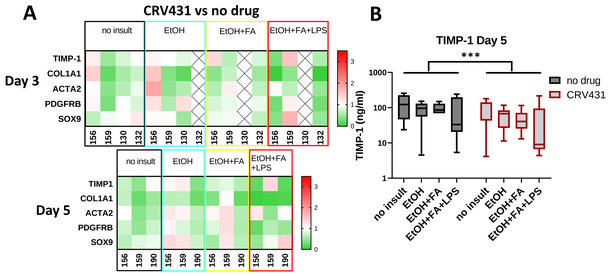


Figure 4: (A) Gene expression of profibrotic markers in PCLS treated with hepatotoxic insults and CRV431 measured by the Quantigene Plex Assay. **(B)** TIMP-1 release in PCLS culture supernatants. n(samples)=9, statistical analysis: 2-way ANOVA model adjusted by subject and condition, p=0.001.

Proinflammatory cytokines were restored to the basal level with CRV431 treatment

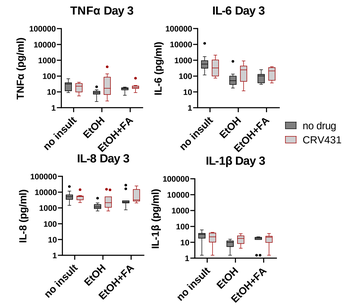


Figure 5: Release of proinflammatory cytokines in PCLS culture supernatants in the presence of the indicated hepatotoxic insults with and without CRV431. n(samples)=13

CRV431 decreased expression of profibrogenic markers in TGFβ stimulated HSC

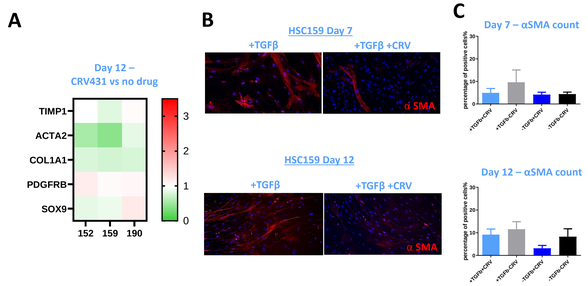


Figure 6: (A) Heatmap showing differential gene expression of activation markers on HSC from 3 subjects treated with TGFβ alone or in combination with CRV431. **(B)** Representative images of αSMA immunostaining (in red, DAPI in blue for nuclear counter stain) on HSC159 treated with TGFβ alone or with TGFβ+CRV at time points day 7 and day 12. **(C)** Quantification of αSMA positive cells (over the total of DAPI+ cells) in cells treated with TGFβ alone or in combination with CRV at time points day 7 and day 12. n=3 cell lines, mean ±SEM.

CRV431 reduced ECM production by TGFβ stimulated HSC

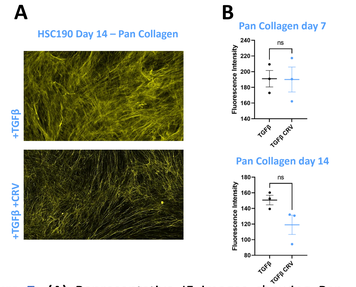


Figure 7: (A) Representative IF images showing Pan-Collagen staining of ECM fibers deposited by HSC159 treated with TGFβ alone or with TGFβ+CRV. **(B)** Quantification of Pan-Collagen via IF (measure of intensity of fluorescence) in HSCs treated with TGFβ or with TGFβ+CRV at the indicated timepoints. n= 3 cell lines; 6 pics/condition MEAN±SEM; statistical analysis: Wilcoxon-Mann-Whitney Test.

CRV431 significantly altered the orientation of ECM fibers

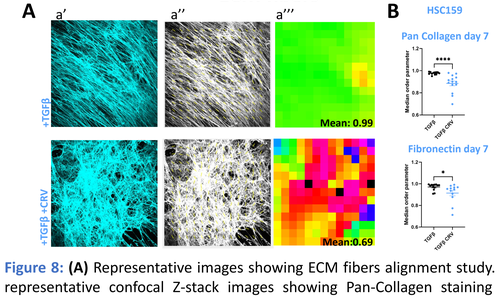


Figure 8: (A) Representative images showing ECM fibers alignment study. a', representative confocal Z-stack images showing Pan-Collagen staining of ECM fibers deposited by HSC159 treated with TGFβ alone or with TGFβ+CRV. a'', segments (in yellow) produced by analysis software AFT – *Alignment by Fourier Transform* (3); each segment is oriented in the same direction as ECM fibers. a''', heatmap is then generated according to fibers orientations, showing diversity of neighbour fibers orientation. **(B)** Mean order parameter of Pan-Collagen and Fibronectin fibres alignment in HSC159-deposited ECM after 7 days of treatment with TGFβ or TGFβ+CRV. n=13-14 pics/condition. mean±SEM; statistical analysis: Wilcoxon-Mann-Whitney Test

Conclusions

- CRV431 was not hepatotoxic and did not induce cell death; it profoundly reduced the expression and secretion of pro-fibrogenic markers and restored a balanced cytokine profile in ALD-PCLS model.
- In HSCs, CRV431 reduced αSMA and collagen deposition/expression when added simultaneously or after TGF-β1 activation and altered the alignment of collagen and fibronectin fibers.
- Our results confirm the role of cyclophilins in liver fibrosis, including HSC activation, collagen deposition and orientation.
- These data reveal the potential for the cyclophilin inhibitor CRV431 to reduce ALD-induced fibrosis and suggest the possibility of using this drug as a therapy in ALD.

References

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