ROCK2 inhibitors for the treatment of NASH

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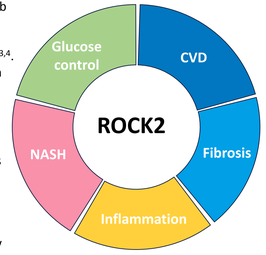
INTRODUCTION

ROCK2 is central to disease processes driving fibrosis pathology

- ROCK2+/- on HFD protected against loss of insulin sensitivity & protected from heart abnormalities[1].
- ↑ROCK activity in vascular endothelium of db/db mice[2].
- ↑ROCK2 in vascular endothelium diabetic animals, ↑ hypertension & vascular dysfunction[3,4].
- ROCK2+/- haplotype mouse showed attenuation of diabetes induced hypertension[6].



- ↑ROCK2 arterial smooth muscle cells, cultured from patients with PAH[5].
- ROCK2 conditional KO protected against hypoxia induced hypertension[5].
- Cardiomyocyte specific ROCK2 KO mice protected against angiotensin induced hypertrophy[7].
- ROCK2-/- bone marrow reduced atherosclerosis on high cholesterol diet[8].

- ↑ROCK2 in liver of db/db mice[9] and liver fibrosis models[10,11].
- ↑ROCK2 signalling drives HSC activation and portal hypertension[9,10,12] and blocking this pathway reduces fibrosis[11].
- ROCK2 implicated in non-canonical Wnt pathway induced steatohepatitis[13].

- ROCK inhibitors reduce fibrosis severity in lung[18], heart[19], and liver fibrosis models[10,11].
- ROCK inhibition reduces fibrosis parameters and albuminuria in kidney fibrosis models[14,15,16].
- ROCK2+/- protected from bleomycin induced lung fibrosis[20].
- ROCK2 inhibitor reduces fibrosis in human cGVHD.

- ↑ROCK2 in acute inflammation in the kidney[17].
- ROCK inhibition reduces pro-inflammatory cytokine release and leukocyte recruitment[14,16,17].

NOTE: *ROCK1 and ROCK2 expression in the vascular endothelium is responsible for the control of vascular tone and systemically active pan-ROCK inhibitors have been shown to induce a drop in arterial blood pressure leading to a corresponding increase in heart rate[21]. We hypothesise that targeting only ROCK2 will not induce hypotension.*

RESULTS

Redx's ROCK2 inhibitors are potent and highly selective

- REDX10178 and REDX10616 are potent and highly selective ROCK2 inhibitors.
- Cellular potency of ROCK2 selective inhibitors is determined by measuring inhibition of pMYPT1, a substrate downstream of ROCK, in MCF7 cell lines; ROCK1 or ROCK2 selective cell lines were generated with shRNA.

ASSAY	REDX10178 IC_{50}	REDX10616 IC_{50}
Biochemical ROCK2 activity	0.002 µM	0.004 µM
Biochemical ROCK1 activity	0.9 µM	1.0 µM
Cellular ROCK2 selective pMYPT1	0.9 µM	1.0 µM
Cellular ROCK1 selective pMYPT1	20 µM	> 30 µM

Table 1. ROCK2 selective tool compounds are active in biochemical and cellular assays.

pMYPT1 mechanistic assay

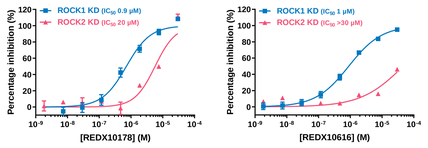
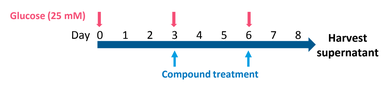


Figure 1. Inhibition of pMYPT1 in ROCK1 or ROCK2 knockdown cell lines. ROCK2 selective compounds are more potent in ROCK1 knockout lines due to compensation of ROCK1 signalling in the wildtype parental lines. Data are from n>3.

ROCK2 inhibitors prevent the release of pro-inflammatory and pro-fibrotic factors in kidney mesangial cells grown in high glucose

- Protein expression of CTGF, fibronectin, PDGF-BB, TIMP-1, MMP-2 and MCP-1 detected in the culture media.

A: Schematic of the experiment



ASSAY	REDX10178	REDX10616
CTGF assay – WB	0.4 µM	0.4 µM
Fibronectin ELISA	0.4 µM	0.2 µM
Secreted TIMP-1 – ELISA	0.2 µM	0.9 µM
Secreted PDGF-BB – ELISA	0.2 µM	0.4 µM
Secreted MCP-1 – ELISA	0.3 µM	1.3 µM
Secreted MMP-2 – ELISA	1.2 µM	2.2 µM

Table 2. Summary of analysis of culture supernatant from cells cultured with Redx's ROCK2 inhibitors. Data are from n≥3.

B: CTGF expression in culture supernatant

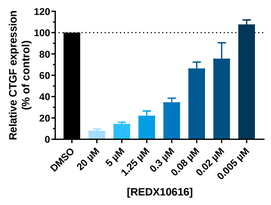


Figure 2. Mouse mesangial cells cultured for 8 days in high glucose with media refresh and compound addition on day 3 and media and compound refreshed on day 6 (A). Supernatant harvested for analysis, representative data in (B).

RESULTS

ROCK2 inhibitors reduce markers of fibrosis in human liver *in vitro* models

- Redx ROCK2 inhibitors reduce primary human hepatic stellate cell activation and and fibrotic gene expression *in vitro*.
- Redx ROCK2 inhibitors reduce lipid accumulation in primary human hepatocytes *in vitro*.

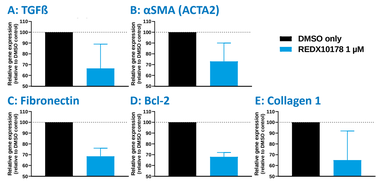


Figure 3. Primary HSC activated with TGFβ for 5 days then treated with REDX10178 for 1h. Gene expression relative to RPS18 normalised to DMSO controls.

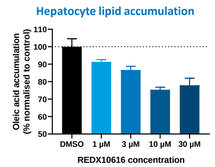
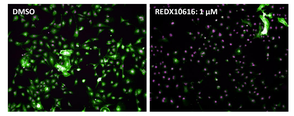


Figure 4. Primary human hepatocytes dosed with REDX10616 plus oleic acid at 1 mM. After 48 h cells labelled with Nile red probe to stain lipids then analysed by flow cytometry. Accumulation normalised to DMSO controls.

- Selective ROCK2 inhibitors reverse myofibroblast phenotype of activated human HSC.
  - Hepatic stellate cell line (LX2) differentiated on stiff plastic for 2 weeks demonstrates a myofibroblast morphology and phenotype indicated by αSMA protein expression, measured by IHC.
  - Redx ROCK2 inhibitors reduce the expression of αSMA in these cells, indicating suppression of the myofibroblast phenotype.

A: αSMA expression in HSC myofibroblasts



Green: αSMA; purple: nuclei (DRAQ5)

B: Quantification of αSMA expression

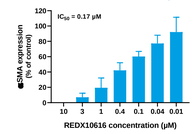


Figure 5. REDX10616 reduces αSMA expression in LX-2 differentiated myofibroblasts. Myofibroblasts expressing αSMA are dosed with compound for 48 h and αSMA expression detected by IHC. (A) Representative images shown where green indicates αSMA and purple nuclei stain (DRAQ5). In (B) mean quantification of αSMA expression in cells dosed with REDX10616 (n=4).

REDX10178 and REDX10616 are highly selective and are suitable probes for *in vivo* studies

REDX10178 kinase panel at 1 µM

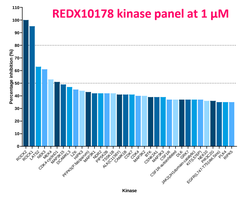


Figure 6. Waterfall plot analysis of kinase panel of 468 kinases for REDX10178 at 1 µM (cut-off at 35% inhibition).

REDX10616 kinase panel at 10 µM

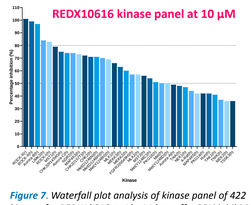


Figure 7. Waterfall plot analysis of kinase panel of 422 kinases for REDX10616 at 10 µM (cut-off a 35% inhibition).

Mouse PK parameters	REDX10178			REDX10616		
Route	IV	PO	PO	IV	PO	PO
Dose (mg/kg)	1	10	30	2	10	50
C_{max} (µM)	1.9	4.6	13	5.2	7.6	55.7
PPB % Fraction unbound (Fu)	5.7	5.7	5.7	8.8	8.8	8.8
$C_{max,\ unbound}$ (µM)	0.01	0.03	0.08	0.05	0.07	0.49
T_{max} (h)	-	0.5	2.0	-	2.0	0.5
$t_{1/2}$ (h)	1.7	4.0	3.5	3.6	1.2	2.2
V_{dss} (L/kg)	0.8	-	-	1.2	-	-
CL (mL/min/kg)	5.3	-	-	14	-	-
% LBF	3.0	-	-	20	-	-
AUC_{0-t} (h.µM)	6.3	26	74	5.1	29	397
$AUC_{0-t,\ unbound}$ (h.µM)	0.04	0.2	0.4	0.05	0.3	3.5
Bioavailability (%)	-	55	39	-	> 100	> 100

Table 3. Pharmacokinetic parameters in C57Bl/6 mice.

- Highly selective ROCK2 inhibitors across a panel of kinases.
- 1 target > 50% inhibition in CEREP SafetyScreen44™.

REDX10178 and REDX10616 suppress inflammatory, fibrosis and kidney injury pathways in a model of acute kidney injury

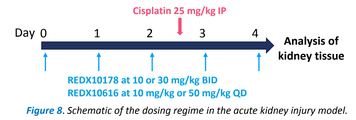


Figure 8. Schematic of the dosing regime in the acute kidney injury model.

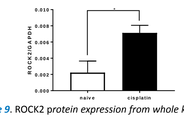
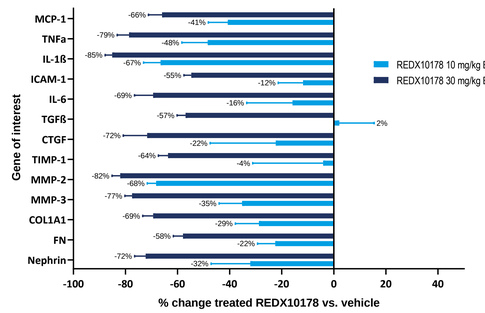


Figure 9. ROCK2 protein expression from whole kidneys.

- Mice treated for 5 days with compound orally, QD or BID. Single IP injection of cisplatin on day 3 induces an acute inflammatory infiltrate.
- This inflammation and injury response in the kidney leads to an increase in the expression of ROCK2 but not ROCK1.
- REDX10178 and REDX10616 modify the expression of genes associated with inflammation, fibrosis and kidney injury in a dose dependent manner.

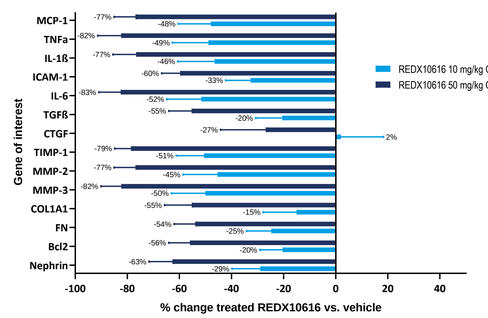
Pro-inflammatory and pro-fibrotic gene expression change following REDX10178 treatment (BID)



P value summary table

P-Value	10 mg/kg BID	30 mg/kg BID
MCP-1	0.01	0.0002
TNFα	0.08	0.009
IL-1β	0.005	0.0008
ICAM-1	0.4	0.002
IL-6	0.6	0.02
TGFβ	0.9	0.0003
CTGF	0.4	0.008
TIMP-1	0.9	0.02
MMP-2	0.004	0.001
MMP-3	0.3	0.02
Collagen 1	0.3	0.02
Fibronectin	0.1	0.0005
Nephrin	0.1	0.001

Pro-inflammatory and pro-fibrotic gene expression change following REDX10616 treatment (QD)



P value summary table

P-Value	10 mg/kg QD	50 mg/kg QD
MCP-1	0.05	0.003
TNFα	0.09	0.006
IL-1β	0.09	0.008
ICAM-1	0.10	0.005
IL-6	0.07	0.007
TGFβ	0.2	0.004
CTGF	0.9	0.2
TIMP-1	0.03	0.002
MMP-2	0.07	0.003
MMP-3	0.08	0.006
Collagen 1	0.4	0.003
Fibronectin	0.09	0.001
Bcl2	0.2	0.003
Nephrin	0.2	0.01

SUMMARY

- Redx have developed a series of compounds that are potent ROCK2 inhibitors in biochemical & cellular *in vitro* assays.
- These compounds are highly selective against ROCK1 and a panel of kinases.
- Targeting ROCK2 selectively allows a safe cardiovascular profile, as previously demonstrated in telemetered rats.[22]
- Selective ROCK2 inhibitors reverse myofibroblast phenotype of activated human hepatic stellate cell myofibroblasts and reduce markers of fibrosis and lipid accumulation in primary HSC and hepatocytes respectively.
- Demonstration that physiologically relevant markers of fibrosis pathways can be modulated *in vivo* with selective ROCK2 inhibitors.
- No safety concerns highlighted from early *in vitro* assessment (hERG, CEREP).
- Redx's highly selective ROCK2 lead compound shows robust preclinical *in vivo* efficacy in murine liver, kidney and lung fibrosis models (undisclosed data).

References: *1. Soliman et al, 2016; 2. Xie et al, 2006; 3. Waddingham et al, 2015; 4. Cicek et al, 2013; 5. Shimizu et al, 2013; 6. Yao et al, 2013; 7. Okamoto et al, 2013; 8. Zhou et al 2012; 9. Hu et al. 2018; 10. Luo et al, 2012; 11 Zhang et al 2016; 12. Trebicka et al, 2007; 13. Wang et al, 2018; 14. Kolavennu et al. 2008; 15. Baba et al. 2014; 16. Sun et al. 2006; 17. Nozaki et al 2015; 18. Zhou et al 2013; 19. Ho et al. 2012; 20. Knipe et al 2015; 21.* Kast *et al, 2017; 22. Flynn et al, 2016. 22.* Guisot *et al,* 3rd NASH Summit, 2019