ROCK2 inhibitors for the treatment of chronic kidney disease

Emily P. Offer, Nicolas E.S. Guisot, Stuart A. Best, Thomas Pesnot, Philip MacFaul, Sara Ceccarelli, Kay Eckersley, Matthew R. Box, Neil Hawkins, Gary R. Pitt, Peter R. Bunyard, Clifford D. Jones, Richard Armer.

Redx Pharma Plc, Alderley Park, Alderley Edge, United Kingdom.

INTRODUCTION

- The Rho Associated Coiled-Coil Containing Protein Kinase (ROCK) serine/threonine kinases, ROCK1 and ROCK2, are central signalling proteins that regulate a range of cellular responses.
- These processes are central to the aberrant wound healing response that can progress to chronic injury and organ fibrosis.
- Small molecule pan-ROCK inhibitors have been shown to be anti-fibrotic in a range of kidney fibrosis *in vivo* models including: STZ induced[1] and db/db type 2 diabetic animal models[2,3], UUO[4,5,6], ischemia refusion injury[7] and hypertension induced injury[8].
- ROCK signalling is also involved in regulating vascular tone and pan-ROCK inhibitors have been shown to cause hyperaemia and hypotension, limiting their use in patients.
- There is evidence that ROCK2 is upregulated in diabetic kidney disease and in the diseased vascular network of patients at risk of chronic kidney disease (CKD).

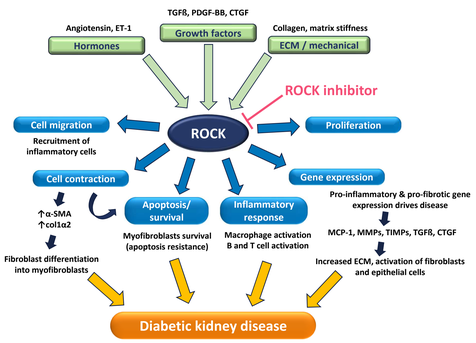


Figure 1. *ROCK is a central node in many pathways associated with tissue injury and fibrosis.*

RESULTS

Redx's ROCK2 inhibitors are potent and highly selective

- REDX10178 and REDX10325 are potent and highly selective ROCK2 inhibitors:
  - High selectivity versus ROCK1 in kinase activity assay.
  - Excellent selectivity across a panel of 468 kinases.
  - Active in cellular mechanistic and disease relevant phenotypic assays.
  - REDX10178 displays good selectivity in the CEREP safety panel with no off target activities observed.

ASSAY	KD025 IC_{50}	REDX10178 IC_{50}	REDX10325 IC_{50}
ROCK2 activity	70 nM	1.4 nM	0.65 nM
ROCK1 activity	5.1 µM	0.1 µM	0.3 µM
Cellular ROCK2 selective pMYPT1	1 µM	0.8 µM	0.2 µM
Cellular parental MCF7 pMYPT1*	0.9 µM	3.9 µM	> 30 µM
Cellular ROCK1 selective pMYPT1*	0.8 µM	8.8 µM	> 30 µM

Table 1. *ROCK2 selective tool compounds are active in biochemical and cellular assays and selective in kinase panel. Comparison with KD025 – Kadmon's ROCK2 selective compound. *Note: Selective ROCK2 compounds expected to be less active in parental and ROCK1 selective MCF7 assays.*

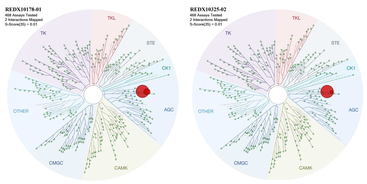
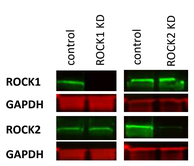


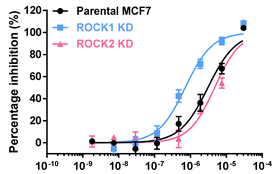
Figure 2. *Kinase selectivity data for ROCK2 inhibitors tested at 1 µM against 468 kinases. Selectivity score (S35) 0.01.*

- Cellular potency of ROCK2 selective inhibitors determined by measuring inhibition of pMYPT1, a substrate downstream of ROCK in MCF7 cell lines.
- To determine ROCK2 selectivity, ROCK1 or ROCK2 selective cell lines were generated with shRNA and potency was compared in these cell lines and compared to wildtype MCF7 cells (*see Table 1*).

A: ROCK expression in MCF7 cell lines



B: REDX10178 inhibition of pMYPT1



C: REDX10325 inhibition of pMYPT1

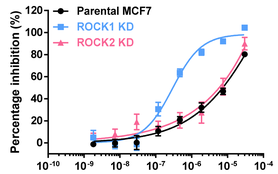


Figure 3. *Inhibition of pMYPT1 in parental and ROCK1 or ROCK2 knockdown cell lines. (A) Western blot showing expression of ROCK1 and ROCK2 following shRNA knockdown. (B,C) ROCK2 selective compounds are more potent in ROCK1 knockout lines due to compensation of ROCK1 signalling in the wildtype parental lines. Data are from n>4.*

RESULTS

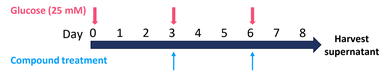
REDX10178 has suitable ADME properties and is orally bioavailable

- Low to moderate *in vitro* clearance across species.
- The % free of REDX10178 in mouse and rat plasma is similar to human (5.7, 4.8% and 6.6% respectively).
- Rat PK: REDX10178 at 100 mg/kg - systemic free drug exposure 1-1.6 fold over ROCK2 pMYPT1 IC_{50} for ~10 h.
- Mouse PK: REDX10178 at 30 mg/kg - systemic free drug exposure 0.2-0.8 fold over ROCK2 pMYPT1 IC_{50} for ~10 h and 0.8-3.1 fold over TIMP-1 IC_{50} for ~10 h.

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 and MCP-1 detected in the culture media.

A: Schematic of the experimental conditions



ASSAY	KD025 IC_{50}	REDX10178 IC_{50}	REDX10325 IC_{50}
CTGF assay – WB	Inactive	0.1 µM	0.4 µM
Fibronectin ELISA	Induction	0.4 µM	0.4 µM
Secreted TIMP-1 – ELISA	0.9 µM	0.2 µM	0.2 µM
Secreted MCP-1 – ELISA	2.9 µM	0.3 µM	0.2 µM
Secreted PDGF-BB – ELISA	10 µM	0.2 µM	0.2 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

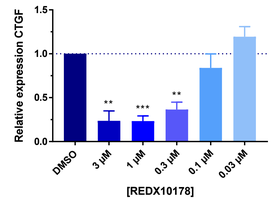
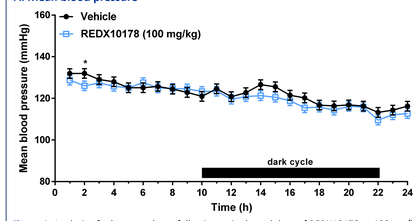


Figure 5. *Mouse mesangial cells cultured for 8 days in high glucose with compound addition on day 3 and media refresh on day 6 (A). Culture supernatant harvested for protein analysis, representative data in (B).*

Selective ROCK2 inhibitor REDX10178 does not induce hypotension or increase heart rate in telemetered rats

- Pan-ROCK inhibitors cannot be safely dosed systemically as they induce hypotension and increased heart rate[9].
- In this study, telemetered rats were dosed with 100 mg/kg REDX10178 to determine whether systemic exposure of a ROCK2 selective compound avoids these CV effects.
- Blood pressure, heart rate, temperature and activity were monitored over 24 h post dose.
- REDX10178 had minimal effect on any parameters tested in this study.

A: Mean blood pressure



B: Heart rate

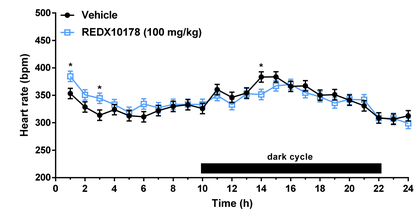


Figure 4. *Analysis of telemetered rats following a single oral dose of REDX10178 at 100 mg/kg (systemic free exposure over pMYPT1 IC_{50} for ~10 h). Crossover study design with two telemetry sessions. Data are plotted LS mean±SEM n=6 animals. Statistical effect of treatment analysed by one way ANOVA with Fisher's LSD post test, compared to vehicle treated animals, *p<0.05.*

- With REDX10178, at early timepoints, the maximum mean blood pressure change from vehicle was 5 mmHg (4%) and 10-20 bpm (4-8%) change in heart rate.
- In comparison, a systemically dosed pan-ROCK inhibitor, with a similar level of free drug cover as REDX10178, resulted in a ~40% drop in blood pressure and ~30% increase in heart rate[9].
- This study suggests that, unlike pan-ROCK inhibitors, systemic exposure of ROCK2 selective drugs could have a clinically acceptable cardiovascular safety profile.

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

- Mice treated for 5 days with compound orally, BID. Single IP injection of cisplatin on day 3 induces an acute inflammatory infiltrate.
- This inflammatory response induces injury in the kidney and leads to an increase in the expression of ROCK2.
- REDX10178 modifies the expression of genes associated with inflammation and fibrosis in a dose dependent manner.

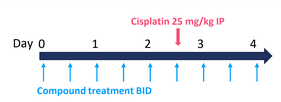


Figure 6. *Schematic of the dosing regime in the acute kidney injury model.*

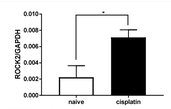


Figure 7. *ROCK2 protein expression from whole kidneys.*

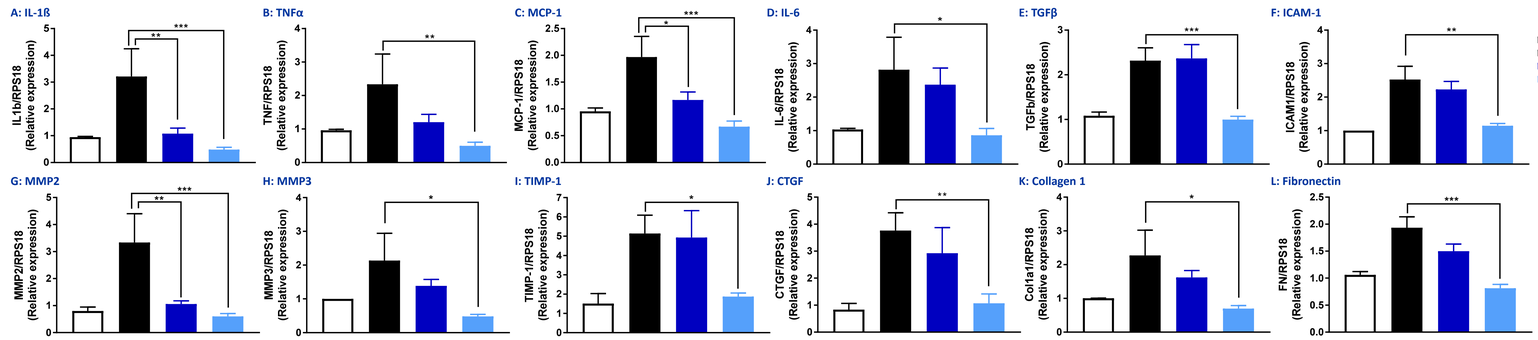


Figure 8. *Gene expression analysis from whole kidneys. Data are plotted mean±SEM n=8 animals. Statistical effect of treatment analysed by one way ANOVA with Fisher's LSD post test, compared to vehicle treated animals, *p<0.05, **p<0.01, ***p<0.001, naive animals excluded from analysis.*

SUMMARY

- Redx have developed potent ROCK2 inhibitors in both biochemical and cellular *in vitro* assays.
- These compounds are highly selective against ROCK1 and a panel of 468 kinases.
- Targeting ROCK2 selectively allows a safe cardiovascular profile, as demonstrated in telemetered rats.
- Demonstration that physiologically relevant markers of fibrosis pathways can be modulated *in vivo* with a selective ROCK2 inhibitor.
- No safety concerns highlighted from early *in vitro* assessment (hERG, CEREP).
- This encouraging profile of tool compound REDX10178 is representative of the potential of the chemical series which are currently in lead optimisation. New compounds with improved profiles are currently under evaluation.
- *In vivo* studies with ROCK2 selective inhibitors in various animal models of fibrosis are ongoing.

1. Peng *et al*; Diabetes 2008 Jun; 57(6): 1683-1692.
2. Kolavennu *et al*; Diabetes. 2008 Mar;57(3):714-23.
3. Matoba *et al*; Int. J. Mol. Sci. 2017, 18(8), 1795.
4. Nagatoya *et al*; Kidney Int. 2002 May;61(5):1684-95.
5. Baba *et al*; Mol Med Rep. 2015 Dec; 12(6): 8010–8020.
6. Fu *et al*; JASN 2006 Nov, 17 (11) 3105-3114.
7. Kentrup *et al*; PLoS One. 2011; 6(10): e26419.
8. Sun *et al*; Br J Pharmacol. 2011 Jan; 162(1): 163–174.
9. Kast *et al*; Br J of Pharmacology. 2007; 152: 1070–1080.

ASN 2018, San Diego; 23rd - 28th October
Poster Number: TH-PO877