Su1552

RDX023-2, a minimally systemic, non-bile acid FXR agonist, reduces steatosis, inflammation and fibrosis in three mouse models of NASH

Andrew J King, Jianhua Chao, Rakesh Jain, Lily Hu, Patricia Finn, Kenji Kozuka, Matthew Siegel, Ying He, Samantha Koo-McCoy, Qumber Jafri, David Rodriguez, Zhengfeng Jiang, Limin He, Jeremy S Caldwell

Ardelyx, Inc., Fremont, CA, USA

Background

- The farnesoid X receptor (FXR) is a ligand-regulated transcription factor highly expressed in the liver and intestine that regulates bile acid, lipid and glucose homeostasis.[1,2]
  - FXR is activated by endogenous bile acids, but can also be modulated by synthetic ligands.
- Systemic FXR agonists have shown therapeutic promise in non-alcoholic steatohepatitis (NASH) but may have adverse effects.[3]
- FXR agonists that target the key pharmacologically responsive tissues (intestine and liver) with minimal systemic exposure may have reduced side effects compared with systemic agonists.
- Here, we characterize RDX023-2, a potent, selective, non-bile acid, minimally systemic FXR agonist, and its effects in three mouse models of NASH.

Methods

Pharmacokinetic and pharmacodynamic studies in wild type mice

- Pharmacokinetic and pharmacodynamic studies were performed in male C57Bl/6 mice following a single oral dose of RDX023-2.
- FXR target gene expression was determined by quantitative polymerase chain reaction (qPCR) with a high dose of LJN452, a potent, systemic FXR agonist, as a positive control. β-actin was used as a reference gene.

Administration of RDX023-2 in three mouse models of NASH

- Six-week-old male C57Bl/6 mice were acclimated on a Western diet (WD; TD.88137, Teklad) for 8 weeks (WD model).
- Six-week-old male *ob/ob* mice were acclimated on a NASH-promoting diet high in trans-fat, cholesterol and simple carbohydrates (D09100301, Research Diets) for 6 weeks (*ob/ob* model).
- Five-week-old male C57Bl/6 mice were acclimated on the NASH-promoting diet (D09100301, Research Diets) and drinking water containing 55:45 fructose:dextrose 42 g/L for 17 weeks (HFCD model).
- RDX023-2 or vehicle (1% methylcellulose in water) was administered once daily by oral gavage for 4 (WD and *ob/ob*) or 6 (HFCD) weeks.
  - LJN452 (systemic FXR agonist) was used as a positive control.
- C57Bl/6 mice fed a standard diet (2018, Teklad) served as healthy controls in the WD and HFCD model; *ob/?* mice were used for the *ob/ob* model. Healthy controls were administered vehicle.

Assessment of the effects of RDX023-2 administration

- Plasma and hepatic lipids, hepatocellular injury enzymes (alanine transaminase [ALT], aspartate transaminase [AST]), fibroblast growth factor (FGF) 15, serum bile acids, and hepatic cytokine and hydroxyproline content were measured.
- Statistical significance was determined by analysis of variance (ANOVA).
- Liver histology was performed by an external blinded pathologist.
- RNA was extracted from liver samples for library preparation and RNA sequencing.

In vitro assays

- FGF19/15 secretion in human and mouse ileum was assessed using primary intestinal epithelial monolayer cultures, as described previously.[4]

Results

Pharmacokinetics and pharmacodynamics

- Oral administration of RDX023-2 (1 mg/kg) resulted in a minimally systemic pharmacokinetic profile (maximum concentration in plasma, 5 ng/mL), with higher maximum drug concentrations measured in the ileum (4470 ng/g) and liver (64 ng/g).
- Administration of RDX023-2 resulted in robust regulation of hepatic and ileal FXR target genes (Figure 1).

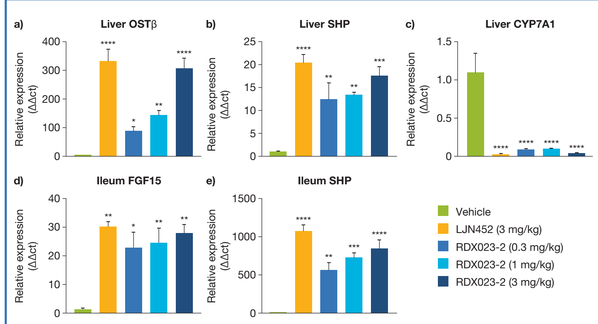
Effects of RDX023-2 in a WD mouse model of hepatic steatosis

- Administration of RDX023-2 for 4 weeks reversed the effects of a WD on hepatosteatosis-related endpoints (Figure 2). RDX023-2:
  - normalized liver triglycerides and liver and plasma cholesterol concentrations at a dose of 1 mg/kg
  - increased plasma concentrations of FGF15 and ileal expression of FXR target genes.

Effects of RDX023-2 in an *ob/ob* mouse model of NASH

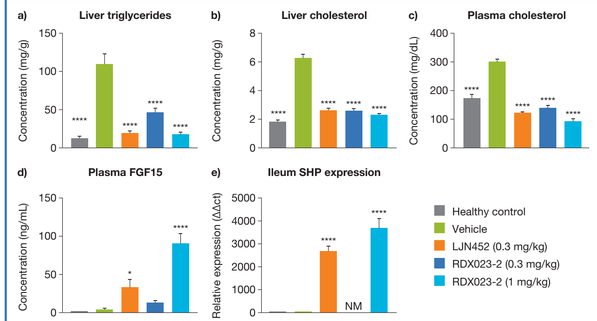
- Administration of RDX023-2 for 4 weeks resolved hepatic steatosis and hepatocellular injury in the *ob/ob* model (Figure 3). RDX023-2:
  - normalized liver triglycerides and plasma cholesterol concentrations and reduced liver cholesterol concentrations at a dose of 1 mg/kg
  - dose-dependently normalized AST and ALT concentrations, indicating reduced hepatocellular injury
  - increased plasma concentrations of FGF15, leading to normalization of serum bile acid levels.

Figure 1. Regulation of FXR target gene expression in the liver and ileum after administration of a single dose of RDX023-2 in wild type mice



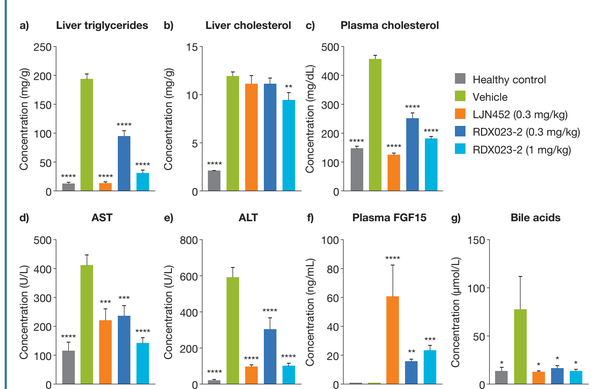
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ versus vehicle.
Data shown are mean values; error bars show standard error (n = 4 per group).
Liver and ileum were harvested for RNA extraction 4 hours after a single dose.
CYP7A1, cytochrome P450 7A1; FGF, fibroblast growth factor; FXR, farnesoid X receptor; OSTβ, organic solute transporter β; SHP, small heterodimer partner.

Figure 2. Effects of RDX023-2 on hepatic steatosis induced by WD feeding



*$p < 0.05$; ****$p < 0.0001$ versus vehicle.
Data shown are mean values after 4 weeks; error bars show standard error (n = 8 per group).
FGF, fibroblast growth factor; NASH, non-alcoholic steatohepatitis; NM, not measured; SHP, small heterodimer partner; WD, Western diet.

Figure 3. Effects of RDX023-2 on hepatic steatosis and hepatocellular injury in an *ob/ob* model of NASH



*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ versus vehicle.
Data shown are mean values after 4 weeks; error bars show standard error (n = 10 per group).
ALT, alanine transaminase; AST, aspartate transaminase; FGF, fibroblast growth factor; NASH, non-alcoholic steatohepatitis.

Effects of RDX023-2 in the HFCD mouse model of NASH

- Administration of RDX023-2 for 6 weeks resolved hepatic steatosis and hypercholesterolemia in the HFCD mice (Figure 4). RDX023-2:
  - normalized liver triglycerides and liver and plasma cholesterol concentrations at a dose of 1 mg/kg
  - normalized AST and ALT concentrations and increased plasma concentrations of FGF15, leading to normalization of serum bile acid levels.
- Administration of RDX023-2 decreased liver concentrations of pro-inflammatory cytokines and the fibrosis marker hydroxyproline, which were elevated in HFCD mice compared with non-diseased controls (Figure 5).
- Liver histology showed that RDX023-2 decreased both macrovesicular and microvesicular steatosis (Figure 5).

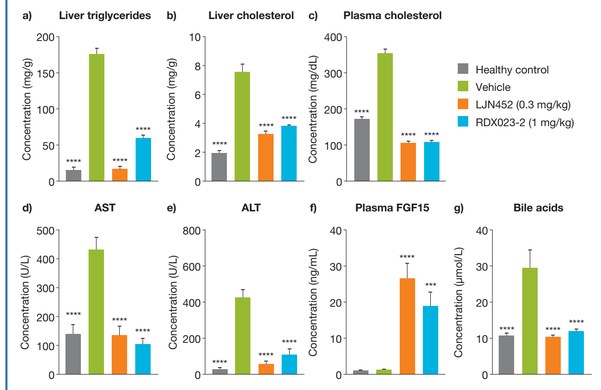
RNA sequencing analysis in *ob/ob* and HFCD mouse models of NASH

- Both *ob/ob* and HFCD mice showed transcriptional dysregulation of lipid metabolism, pro-inflammatory and pro-fibrotic genes, which was attenuated by treatment with RDX023-2 (Figure 6).

Effects of RDX023-2 in an *in vitro* cellular model of human ileum

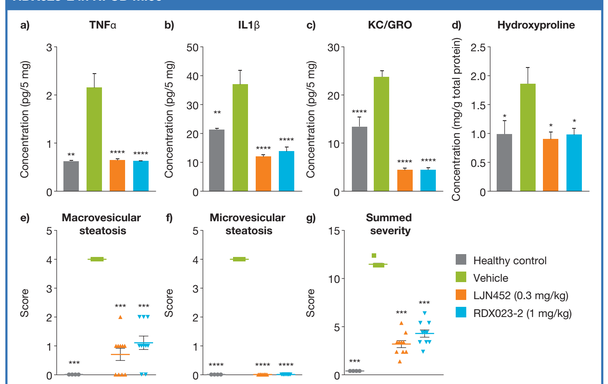
- RDX023-2 showed similar potency in human and mouse translational FGF secretion assays (Figure 7).

Figure 4. Effects of RDX023-2 on hepatic steatosis and hypercholesterolemia in the HFCD model of NASH



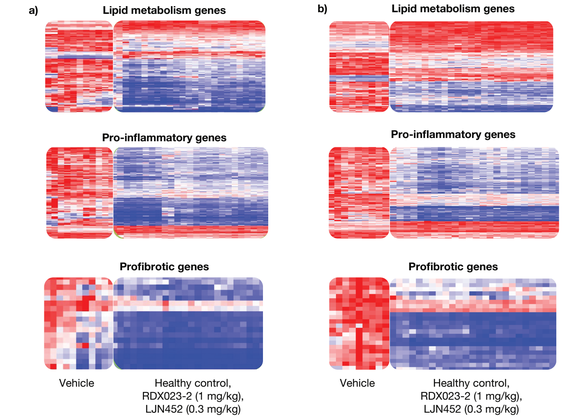
*$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$ versus vehicle.
Data shown are mean values after 6 weeks; error bars show standard error (n = 10 per group).
ALT, alanine transaminase; AST, aspartate transaminase; FGF, fibroblast growth factor; NASH, non-alcoholic steatohepatitis.

Figure 5. Decreased liver inflammation, fibrosis and steatosis after administration of RDX023-2 in HFCD mice



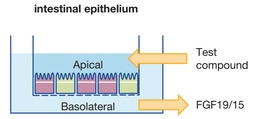
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ versus vehicle.
Data shown are mean values after 6 weeks; error bars show standard error (n = 10 per group).
Histology scoring: not present = 0, minimal = 1, mild = 2, moderate = 3, marked = 4; summed severity = combined individual scores for each criteria per individual animal.
IL1β, interleukin 1β; KC/GRO, keratinocyte chemoattractant/growth-regulated oncogene; NASH, non-alcoholic steatohepatitis; TNFα, tumor necrosis factor α.

Figure 6. Normalization of liver gene expression patterns after administration of RDX023-2 1 mg/kg in a) *ob/ob* and b) HFCD mice



Unsupervised clustering of enriched GO pathways central to the pathogenesis of NASH (lipid metabolism, GO:0006629; pro-inflammatory, GO:0002376; profibrotic, GO:0030199).
GO, gene ontology; NASH, non-alcoholic steatohepatitis.

Figure 7. FGF secretion assay in human and mouse intestinal epithelial stem cell monolayers



	RDX023-2
Human ileum FGF19	
EC_{50}, nM	5
Efficacy, %	100
Mouse ileum FGF15	
EC_{50}, nM	3.6
Efficacy, %	96

EC_{50}, half maximal effective concentration; FGF, fibroblast growth factor.

Conclusions

- RDX023-2 is an efficacious, minimally systemic, non-bile acid FXR agonist that effectively reduced hepatic steatosis in three mouse models of NASH.
  - Effects on hepatosteatosis-related endpoints were comparable to those elicited by a potent, systemic FXR agonist.
  - Administration of RDX023-2 was associated with reduced liver inflammation and fibrosis, and normalization of liver gene expression patterns.
- The effects of RDX023-2 on basolateral FGF19 secretion in an *in vitro* model of the human ileum suggest translational potential.
- These results suggest that a minimally systemic FXR agonist such as RDX023-2 could be useful for the treatment of patients with NASH, with the potential for fewer side effects than systemic FXR agonists.

References

1. Matsubara T *et al. Mol Cell Endocrinol* 2013;368:17–29.
2. Shaik FB *et al. Inflamm Res* 2015;64:9–20.
3. Neuschwander-Tetri BA *et al. Lancet* 2015;385:956–65.
4. Kozuka K *et al. Stem Cell Rep* 2017;9:1976–90.

Acknowledgments

Medical writing support was provided by Sarah Graham, PhD, of PharmaGenesis London, London, UK and funded by Ardelyx.

Disclosures

This study was funded by Ardelyx. All authors are current or former employees of Ardelyx.

Presented at Digestive Disease Week, June 2–5, 2018, Washington, DC, USA