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Inhibition of gastrointestinal (GI) NHE3 normalizes GI transit in models of opioid-induced constipation, multiple sclerosis and cystic fibrosis

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Background

- Sodium/hydrogen exchanger 3 (NHE3) plays a prominent role in luminal sodium and water absorption from the gastrointestinal (GI) tract.[1]
- Tenapanor, a first-in-class, minimally systemic, small-molecule inhibitor of NHE3, inhibits absorption of GI sodium and phosphate.[2,3]
- In patients with constipation-predominant irritable bowel syndrome, tenapanor increases complete spontaneous bowel movement responder rate and reduces abdominal pain.[4]
- Here, we characterize a second-generation, minimally systemic NHE3 inhibitor, RDX011-1, and investigate its effects in rodent models of multiple sclerosis (MS), opioid-induced constipation (OIC), and cystic fibrosis (CF), all characterized by impaired GI transit.

Methods

In vitro characterization of RDX011-1

- The potency of RDX011-1 was assessed in:
  - OK cells transiently transfected with rat NHE3
  - PS120 cells stably transfected with sodium/hydrogen exchange regulatory cofactor 2 and human NHE3.
- NHE3 activity was assayed using BCECF, a pH-sensitive dye, to measure the rate of recovery from intracellular acidification:
  - when cells were in the presence of RDX011-1 following incubation for 10 min (pre-incubation assay)
  - after cells were incubated with RDX011-1 for 60 min and washed for 40 min (persistence assay).
- RDX011-1 potency was also assessed in primary human intestinal epithelial monolayer cultures grown in Transwells using BCECF to measure NHE3-mediated apical proton secretion, as described previously.[5]

Pharmacodynamics and pharmacokinetics of RDX011-1 in rats

- Male Sprague Dawley (SD) rats were given a single oral dose of RDX011-1 0.017, 0.06 or 0.17 mg/kg or vehicle (n = 5/group), and housed individually in metabolic cages. Urine was collected for 12 h after dosing to assess intestinal sodium absorption. Fecal form score (1–5) was also assessed 12 h post-dose.
- Male SD rats were given a single oral dose of RDX011-1 0.1 mg/kg or vehicle (n = 4/group/time point) immediately before a standardized meal. Luminal RDX011-1, sodium and water were measured after 1 h and 2 h in multiple intestinal segments.
- In a separate experiment, SD rats were given either an intravenous (1 mg/kg) or an oral dose (30 mg/kg) of RDX011-1. Blood was collected from the jugular and portal veins and bile was collected from the gallbladder to assess RDX011-1 pharmacokinetics.

Small-intestinal transit in experimental autoimmune encephalomyelitis (EAE) and OIC mice

- EAE was induced in female C57BL/6 mice (n = 10/group) by immunization with myelin-derived antigens to model MS.
  - At the onset of EAE symptoms (post-immunization day 11) mice were fasted overnight and dosed orally with RDX011-1 0.3, 1.0 or 3.0 mg/kg or vehicle, and small-intestinal transit assessed.
- OIC was induced in female C57BL/6 mice (n = 10/group) by subcutaneous injection of the peripherally restricted μ-opioid receptor agonist loperamide at 3 mg/kg.
  - Mice were fasted overnight and dosed orally with RDX011-1 0.3, 1.0 or 3.0 mg/kg or vehicle 15 min before subcutaneous injection of loperamide. Small-intestinal transit was assessed 30 min after loperamide administration.
- Small-intestinal transit was monitored after oral administration of Evans Blue dye (6%, 100 μL) and measurement of the dye front after 35 min using the formula: transit distance of dye front/total length of small intestine (pylorus to ileocecal junction) × 100%.

Whole-gut transit in OIC rats

- OIC was induced in male SD rats (n = 8/group) by twice-daily oral dosing of loperamide 3 mg/kg for 3 days.
- Following OIC induction, animals were dosed orally twice daily with RDX011-1 0.3, 1.0 or 3.0 mg/kg or vehicle, for five doses while loperamide administration continued.
- Whole-gut transit was monitored by orally administering Solvent Green 7 dye (10 mg/mL,1 mL/rat) and recording the time taken for the dye to first appear in the stool.

Small-intestinal transit in CF mice

- Wild-type control (WT) and CF mice (harboring premature stop mutations in *CFTR* and no functional CFTR protein [n = 6–10/group]) were fasted overnight and dosed orally with RDX011-1 0.15, 0.3 or 1.5 mg/kg or vehicle.
- Small-intestinal transit was monitored by orally administering rhodamine-dextran dye (25 mg/mL, 100 μL) 15 min after RDX011-1 or vehicle administration and measuring the geometric center of fluorescence after 45 min.

Survival of CF mice

- Transgenic CF mice harboring premature stop mutations in *CFTR* have high mortality in the post-weaning period due to distal intestinal obstructive syndrome (DIOS).[6]
- RDX011-1 0.5 mg/kg or vehicle was administered orally twice daily to WT and CF mice (n = 12–18/group) from weaning (postnatal day 15) for 20 days to test whether chronic NHE3 inhibition prevented DIOS, evaluated by monitoring survival and assessing GI mucus level and goblet cell number histologically.

Statistical analyses

- Continuous, quantitative parameters were analyzed using one-way analysis of variance, with *post hoc* multiple comparisons of treatment versus vehicle control performed using Dunnett's test. Ordinal parameters were analyzed using the non-parametric Fisher's exact test. A *p* value below 0.05 was considered statistically significant.

Results

In vitro characterization of RDX011-1

- Cellular assays indicated that RDX011-1 is a sub-nanomolar full inhibitor of NHE3 and is equipotent against rat and human NHE3 in pre-incubation and persistence assays (Table 1).
- Similar potencies of RDX011-1 were observed in human intestinal epithelial monolayer cultures, with RDX011-1 inhibiting NHE3-mediated acidification of the apical compartment following overnight incubation (IC_{50}, 0.05 nM).

Table 1. *In vitro* pharmacology of RDX011-1: rat and human cellular NHE3 IC_{50}

Assay	IC_{50}, nM	Efficacy, %
Rat		
Pre-incubation	0.23	85
Persistence	0.57	99
Human		
Pre-incubation	0.1	99
Persistence	0.12	100

IC_{50}, concentration that produces 50% inhibition; NHE3, sodium/hydrogen exchanger 3.

Pharmacodynamics of RDX011-1 in rats

- RDX011-1 dose-dependently:
  - reduced urinary sodium excretion and the fraction of dietary sodium excreted in urine (Figure 1a,b)
  - increased fecal form score, indicating increased dietary sodium and water in stool (Figure 1c).
- RDX011-1 significantly retained dietary sodium and water in the lumen of the GI tract compared with vehicle (Figure 2a, b).

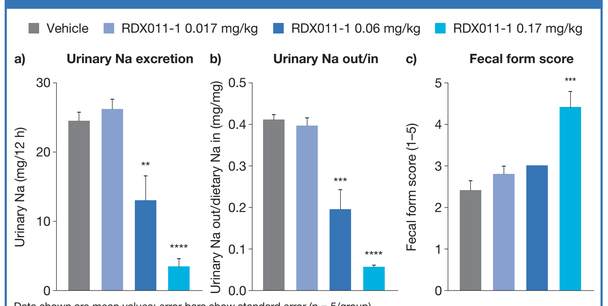
Pharmacokinetics of RDX011-1 in rats

- The maximal luminal concentration of RDX011-1 following an efficacious oral dose was approximately 0.1 μM (Figure 2c).
- RDX011-1 was rapidly cleared following an intravenous dose, and plasma concentrations in the jugular and portal veins and in bile following a high (30 mg/kg) oral dose were extremely low (area under the concentration–time curve, < 49–55 ng.mL/h), indicating very low intestinal absorption of RDX011-1.

Effects of RDX011-1 in rodent models of GI dysmotility diseases

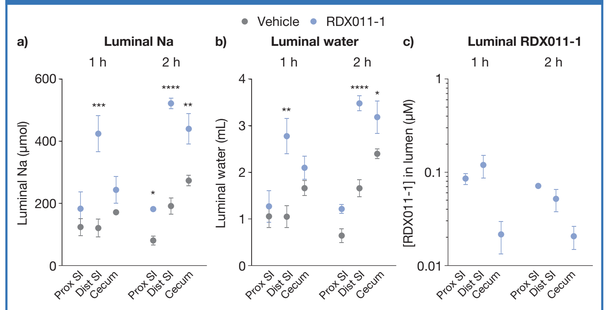
- EAE mice had significantly reduced small-intestinal transit compared with control animals, which was restored by all RDX011-1 doses to control levels (Figure 3).
- OIC mice had significantly reduced small-intestinal transit, and OIC rats had significantly prolonged whole-gut transit time, compared with control animals; both were dose-dependently restored by RDX011-1 to control levels (Figure 4).
- CF mice also had significantly reduced small-intestinal transit compared with WT control mice, which was dose-dependently restored to WT levels by RDX011-1 at all doses (Figure 5a).
- Over the 20-day period immediately post-weaning:
  - CF mice had a high mortality (34% survival)
  - twice-daily oral dosing with RDX011-1 improved survival of CF mice (83%) and reduced intestinal mucus production and goblet cell number towards WT levels compared with vehicle (Figure 5b–d).

Figure 1. RDX011-1 in rats induced dose-dependent reductions in a) urinary sodium excretion and b) the fraction of dietary sodium excreted in urine, and c) increases in fecal form score



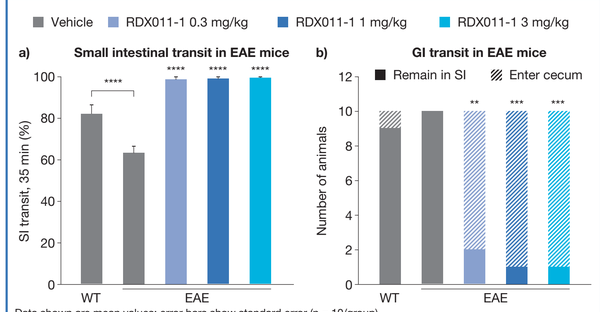
Data shown are mean values; error bars show standard error (n = 5/group).
**$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ versus vehicle.
Na, sodium.

Figure 2. Following RDX011-1 dosing in rats, a) dietary sodium, b) water and c) RDX011-1 itself were significantly retained in the GI tract



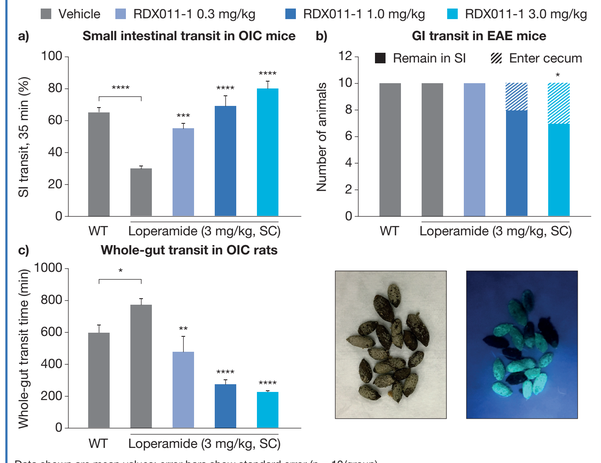
Data shown are mean values; error bars show standard error (n = 4/group/time point).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ versus vehicle.
GI, gastrointestinal; dist, distal; Na, sodium; prox, proximal; SI, small intestine.

Figure 3. RDX011-1 restored 35-min a) small-intestinal transit and b) GI transit in EAE mice



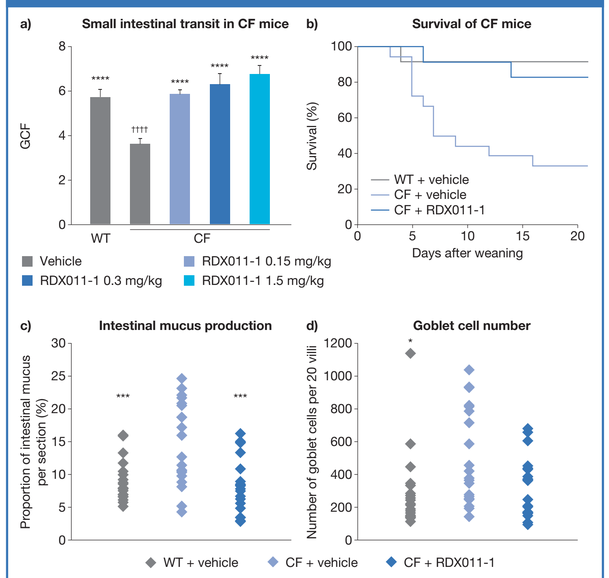
Data shown are mean values; error bars show standard error (n = 10/group).
**$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ versus disease model treated with vehicle.
EAE, experimental autoimmune encephalomyelitis; GI, gastrointestinal; SI, small intestine; WT, wild-type.

Figure 4. RDX011-1 dose-dependently restored a) 35-min small-intestinal transit and b) 35-min GI transit in OIC mice and c) whole-gut transit in OIC rats



Data shown are mean values; error bars show standard error (n = 10/group).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ versus disease model treated with vehicle.
In c), representative images of stools showing the appearance of Solvent Green 7 dye are included.
GI, gastrointestinal; OIC, opioid-induced constipation; SC, subcutaneous; SI, small intestine; WT, wild-type.

Figure 5. RDX011-1 a) restored small-intestinal transit in CF mice, and over the 20-day post-weaning period, reduced: b) mortality of CF mice, c) intestinal mucus production and d) goblet cell number in CF mice



n = 12–18/group.
Data shown in a) are mean values; error bars show standard error
*$p < 0.05$, ***$p = 0.001$, ****$p < 0.0001$ versus CF mice treated with vehicle. ††††$p < 0.0001$ versus WT mice treated with vehicle.
CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; GCF, geometric center of fluorescence; WT, wild-type.

Conclusions

- RDX011-1 is a potent, gastrointestinally restricted inhibitor of NHE3 that effectively and sustainably reduces intestinal sodium and phosphate absorption in rats.
- NHE3 inhibition by RDX011-1 in the GI tract significantly improved GI transit in animal models of OIC, MS and CF.
- In addition, RDX011-1 reduced DIOS-related mortality in CF mice and restored intestinal mucus production and goblet cell number towards WT levels.
- RDX011-1 is a potent prokinetic that has therapeutic potential in diverse diseases that are accompanied by impaired GI motility, including MS, OIC and CF.

References

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Disclosures

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