QR-010, an RNA therapy, restores CFTR function in the saliva secretion assay

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Introduction

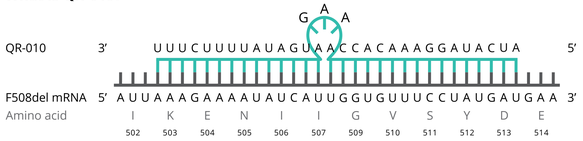
- Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR).
- The most common gene mutation is F508del, causing a deletion of three nucleotides resulting in a non-functional CFTR protein. F508del-CFTR mice have mRNA homologous with human mRNA at the F508del locus.
- QR-010 is an investigational single-stranded chemically modified RNA oligonucleotide designed to be fully complementary to the wild-type (WT) CFTR mRNA. Functional repair of CFTR in F508del-CFTR mice has been reported before by us, using the nasal potential difference assay[1].
- QR-010, is being developed as an inhaled agent. Oro-tracheal (OT) administration was used to mimic inhalation. We therefore investigated whether or not intra-airway delivery of QR-010 resulted in local uptake in the airway epithelium as well as systemic distribution to extrapulmonary organs.
- A saliva secretion assay (SSA), a murine surrogate of the human sweat chloride test, was used to assess restoration of CFTR function in the salivary glands by QR-010. F508del-CFTR mice have little- to no CFTR-mediated saliva production.

Goals & Objectives

- **To assess and compare uptake of QR-010 by the airway epithelium and bio distribution to extra-pulmonary organs after OT or IV administration.**
- **Assess functional repair of CFTR in F508del-CFTR mice after OT administered QR-010 by measuring CFTR-mediated saliva secretion.**

Materials & Methods

What is QR-010?



Sequence of QR-010 with the (G-A-A) representing the complement of the missing C-U-U sequence in the F508del mRNA.

Detection of Cy5-labeled QR-010 in Tissue

- WT (C57BL/6) or F508del-CFTR mice[2] received a single dose of Cy5-labeled QR-010 (10mg/kg) by OT or IV administration.
- Microscopic detection of Cy5 in paraffin embedded lung and salivary gland sections was used to assess localization of Cy5-labeled QR-010.

Quantification of QR-010 in Serum and Tissue

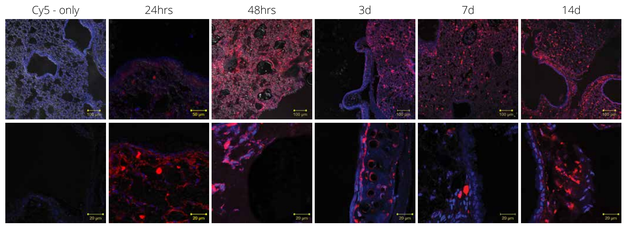
- WT (C57BL/6) mice received a single dose of (unlabeled) QR-010 (10mg/kg) by OT or IV administration.
- Absolute QR-010 concentrations were detected by a hybridization-HPLC method using a fluorescently labeled PNA probe homologous to QR-010 to capture QR-010 in serum and tissue lysates.

Saliva Secretion Assay

- F508del-CFTR mice (12w and older, M/F) received QR-010 (10mg/kg), or saline as control, every other day by OT administration up to 6 doses. Washout was assessed 1, 2, 3 and 4 weeks after the 6th dose.
- A modified method developed by Best et al (2004)[3] was used for the SSA: Mice were anesthetized by isoflurane.
- Subcutaneous injection of atropine to block cholinergic and adrenergic system (1mM) and isoproterenol (100uM) to induce CFTR-mediated saliva production.
- Saliva was absorbed in pre-weighted pieces of filter paper and replaced every 3min for 30 min. Total saliva production was calculated by adding the corrected weight of each filter divided by the bodyweight.

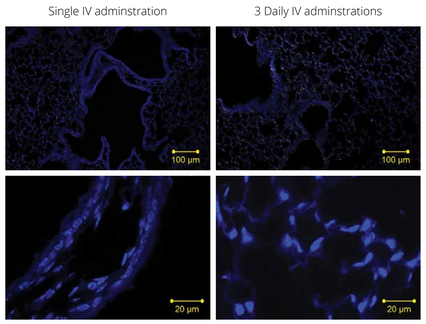
Results

Cy5-labeled QR-010 was visualized in airway epithelium after OT administration



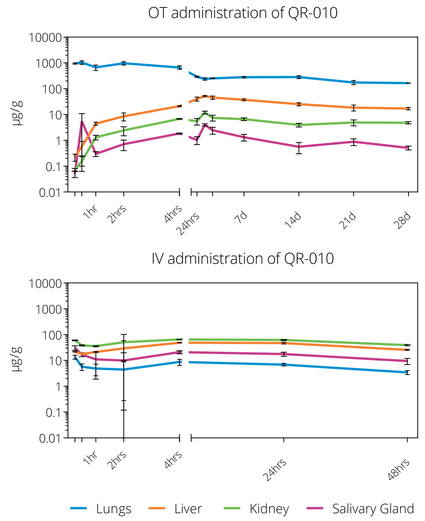
Strong Cy5 signal was visualized in the airway epithelium of WT mice up to 14 days after a single OT administration of Cy5-labeled QR-010 (10mg/kg). Cy5 is shown in red and DAPI in blue.

Cy5-labeled QR-010 was not visualized in airway epithelium after IV administration



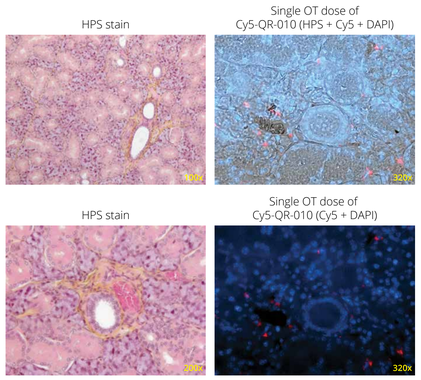
Cy5 signal could not be visualized in the lung airway epithelium after single and multiple (3x) IV doses of Cy5-labeled QR-010 (10mg/kg) in WT mice. Cy5 is shown in red and DAPI in blue.

Uptake of QR-010 by lungs and bio distribution to extrapulmonary organs after OT administration.



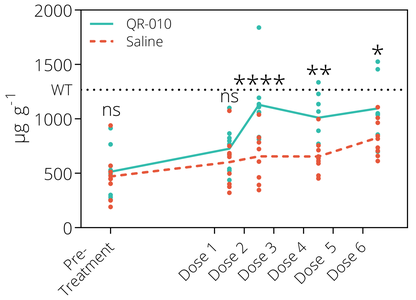
Rapid uptake of QR-010 by the airway epithelium after OT administration (10mg/kg) in WT mice. Lung uptake of QR-010 was greater than uptake in extrapulmonary organs after OT administration compared to IV administration.

Cy5-labeled QR-010 was visualized in the female salivary glands after OT administration



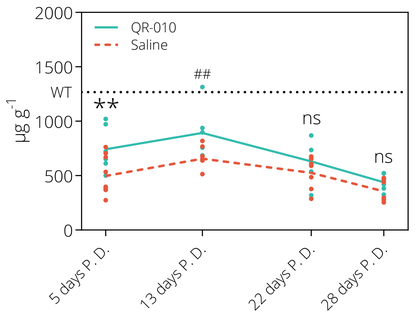
Salivary gland tissue was stained with hematoxylin phloxine saffron (HPS) stain. Cy5 signal was visualized in the Salivary (Submandibular) glands of F508del-CFTR mice after a single dose of Cy5-labeled QR-010 (10mg/kg). Cy5 is shown in red and DAPI in blue.

QR-010 restores CFTR-mediated saliva secretion in female F508del-CFTR mice



Two up to six OT administrations of QR-010 (10 mg/kg) compared to saline significantly improved the CFTR-mediated salivary secretion in female F508del-CFTR mice. Lines show mean CFTR-mediated saliva secretion corrected for bodyweight (µg saliva/g body weight). Dotted straight line represents the saliva secretion in sex- and-age-matched WT mice. Treatment groups per no. of doses were compared by two-way ANOVA with Fisher's LSD test: ns=non-significant, ****p<0.00001, **p=0.0029, *p=0.0233. Repeated measures ANOVA confirmed a signifcant improvement of CFTR-mediated saliva secretion after treatment with QR-010 compared to saline. There was no effect of QR-010 on male F508del-CFTR mice.

Effect of QR-010 on CFTR-mediated saliva secretion remains up to 13 days post-dosing



Effect of QR-010 on CFTR-mediated saliva secretion remains up to 13 days post-dosing (P. D.) Lines show the mean CFTR-mediated saliva secretion corrected for bodyweight. Dotted straight line represents the saliva secretion in sex- and-age-matched WT mice. Treatment groups per no. of days P.D. were compared by two-way ANOVA with Fisher's LSD test: ns=non-significant, **p=0.0038, ##p=0.0076, *p=0.0149.

Discussion

- QR-010 was taken up rapidly by airway epithelium after OT administration.
- Cy5-labeled QR-010 was not visualized in the airway after IV administration, but detectable levels were present in lungs by H-HPLC.
- Stable and high QR-010 levels in lung were detected up to 28 days after OT administration. With Cy5 we see a shift to brighter macrophages and less bright epithelial cells, consistent with airway clearance by phagocytosis..
- Bio distribution to salivary gland after OT administration is similar to IV administration.
- Cy5-labeled QR-010 was visualized in the female salivary gland after a single OT administration of Cy5-labeled QR-010.
- Unsurprisingly, no effect of QR-010 on the CFTR-mediated saliva secretion was found in male F508del-CFTR mice as gender differences have been reported before[4]. This might be caused by distinct morphological differences between male and female salivary glands resulting in a different biodistribution profile[5].
- Functional repair of CFTR after OT administration of QR-010 in extrapulmonary organs in female F508del-CFTR mice was demonstrated in the SSA by increased saliva volume.

Conclusion

QR-010 administered oro-tracheally demonstrates the ability to restore CFTR function in salivary glands of F508del-CFTR mice as measured by increased saliva volume.

References

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Thank you

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