Adeno-associated Viral Vector-mediated shRNA Delivery Strategies to Generate Intestinal and Hepatic Metformin Transporter-knockdown Mouse Models

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Purpose
Metformin is a leading oral anti-diabetic drug used for the treatment of type II diabetes mellitus. Despite being highly hydrophilic and positively charged at all physiological pH values, the oral bioavailability of metformin is high (~50-60%)1.

In vitro studies at the Thakker laboratory using Caco-2 cell monolayers showed that metformin apical (AP) uptake and efflux is efficient, while basolateral (BL) egress is negligible, resulting in predominantly paracellular absorptive transport2. To explain the high bioavailability of metformin, we proposed a hypothesis that AP organic cation-selective transporters (OCT1, PMAT, SERT and CHT) augment the intestinal absorption of metformin via the paracellular route by repeated cycling of the drug between the intestinal lumen and enterocyte3 (Fig 1).

Figure 1. Proposed Intestinal Absorption Mechanism of Metformin

Results
The relative contributions of intestinal and hepatic transporters to metformin disposition and pharmacology can be evaluated using mouse models in which intestine- and liver-specific organic cation transporters are knocked down. RNA interference (RNAi) is the most promising gene silencing pathway, which utilizes siRNA, miRNA or multiple hairpin miRNA (MmiR) to suppress gene expression. As a proof-of-concept, this study aims to determine if siRNA/miRNA/MmiR, packaged in adenovirus-associated viral (AAV) vector, can silence mOct1 gene in mouse intestine and/or liver.

Methods
Generation of shRNA constructs and packaging into AAV:
Three siRNA sequences (siRNA 058, siRNA 059 and siRNA 060) targeting mOct1 mRNA were cloned between BamHI and HindIII downstream of the H1 promoter in the pTR-CBA-Tom AAV plasmid. Single hairpin miRNA (mOct 059 and mOct 060) and MmiR (058-060-060) sequences were cloned into EcoRI and Sall enzyme sites between the CBA promoter and td-tomato gene (Fig 2). Plasmid vectors were transfected into mOct1-expressing Chinese Hamster Ovary (CHO)-K1 cells by electroporation. mOct1 knockdown was evaluated by quantitative real polymerase chain reaction (qRT-PCR). Plasmid vectors with robust knockdown efficiency were individually packaged into liver-specific AAV serotype 8 (Fig 3), and 5 x 1011 viral particles (200 μl) of the AAV vectors were administered to mice via tail vein injections. After two, four and eight weeks of AAV administration, mice were anesthetized and the liver, intestine and kidney were collected. RNA was isolated from these tissues and gene expression of mOct1 was measured by (qRT-PCR).

At two weeks post vector delivery, a trend towards reduced expression of mOct1 mRNA, specifically with MmiR and miR scrambled (SC) sequences was observed in all three tissues (Fig 5A).

However, mOct1 mRNA expression was significantly reduced with siRNA target sequences in all the three tissues at 2 weeks post dosing.

Conclusions
- siRNA targeting mOct1, cloned into pTR-CBA-Tom, successfully knocked down mOct1 mRNA expression in mOct1-expressing CHO-K1 cells.
- Organ-specific knockdown of mOct1 mRNA expression was successfully achieved with miRNAs.
- MmiR caused robust knockdown of mOct1 perhaps because of three repeated hairpin loops, as well as a strong CBA promoter.
- Suppression of mOct1 mRNA expression caused by the miRNA scrambled sequence could be due to interference with the endogenous intracellular miRNA machinery.
- siRNAs failed to knockdown mOct1 mRNA expression in the liver, kidney and intestine, but rather increased expression of mOct1 mRNA was observed.

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References