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Review

RNA interference in the treatment of renal stone disease: Current status and future potentials

Kyle D. Wood*, Ross P. Holmes, John Knight

Department of Urology, 816 KAUL Building, 720 20th Street South, The University of Alabama at Birmingham, Birmingham, AL, 35294-3411, USA

HIGHLIGHTS

- Utilization of lipid nanoparticles and conjugation with *N*-acetyl galactosamine, have allowed liver specific RNAi therapy.
- RNAi targeting glycolate oxidase and/or hydroxyproline dehydrogenase can lower urinary oxalate excretion in animal models.
- Clinical trials are currently ongoing testing RNAi targeting glycolate oxidase in Type 1 Primary Hyperoxaluria subjects.
- Further elaboration of resorptive pathways, specifically involving calcium and citrate, may lead to future targets for RNAi.

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ABSTRACT

Recent advances in RNA interference (RNAi) delivery and chemistry have resulted in the development of more than 20 RNAi-based therapeutics, several of which are now in Phase III trials. The most advanced clinical trials have utilized modifications such as lipid nanoparticles and conjugation to *N*-acetyl galactosamine to treat liver specific diseases. Recent reports have suggested that reducing endogenous oxalate synthesis by RNAi may be a safe and effective therapy for patients with the rare disease, Primary Hyperoxaluria (PH). Our current understanding of endogenous oxalate synthesis indicates that two enzymes, hydroxyproline dehydrogenase and glycolate oxidase (GO), are suitable targets for oxalate reduction therapy. Our studies in a mouse model of PH type 1 have demonstrated that reducing the expression of either of these enzymes in the liver with RNAi significantly reduces urinary oxalate excretion. Early human phase clinical trials are now under way in PH1 patients with RNAi targeting GO. Future elaboration of other contributors of stone disease and improvement in tissue specific targeting with RNAi may lead to further therapies that target idiopathic stone disease.

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1. Introduction

RNAi involves the pairing of a short RNA sequence with a 21-nucleotide endogenous mRNA target (Fig. 1) [1]. First identified in 1998 by Craig Mello and Andrew Fire, endogenous RNAi regulates many cellular processes in plants and animals [2]. Therapeutically, RNAi works via delivery of small RNA duplexes, including microRNA (miRNA) mimics, short interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and Dicer substrate RNAs (dsiRNAs) [3]. These double stranded RNAs interact with RNA-induced silencing complex (RISC) which then unloads one strand leaving a single RNA strand to then interact with the complementary mRNA sequence. This interaction with the mRNA results in degradation and suppression and thus decreased target protein expression [4]. All four types of RNAi are currently in clinical trials to treat a wide spectrum of diseases including cancer, genetic conditions, and infections/pathogens. Much of the effort regarding clinical translation of RNAi has focused on increasing stability, decreasing off-target effects, and optimizing delivery to specific tissues. Recent advances in RNAi delivery, including the use of lipid nanoparticles (LNPs) and *N*-acetyl galactosamine (GalNAc), have made liver-targeting RNAi the forerunners in the race for clinically approved RNAi therapeutics; however, other modifications (aerosolized, direct injection, conjugation, nanotechnology, etc) may allow treatment of diseases arising in a number of other organ systems [3]. LNPs increase stability and optimize delivery of the siRNA to hepatocytes by

* Corresponding author.
E-mail addresses: kwood2@uabmc.edu (K.D. Wood), rholmes@uab.edu (R.P. Holmes), knight74@uab.edu (J. Knight).

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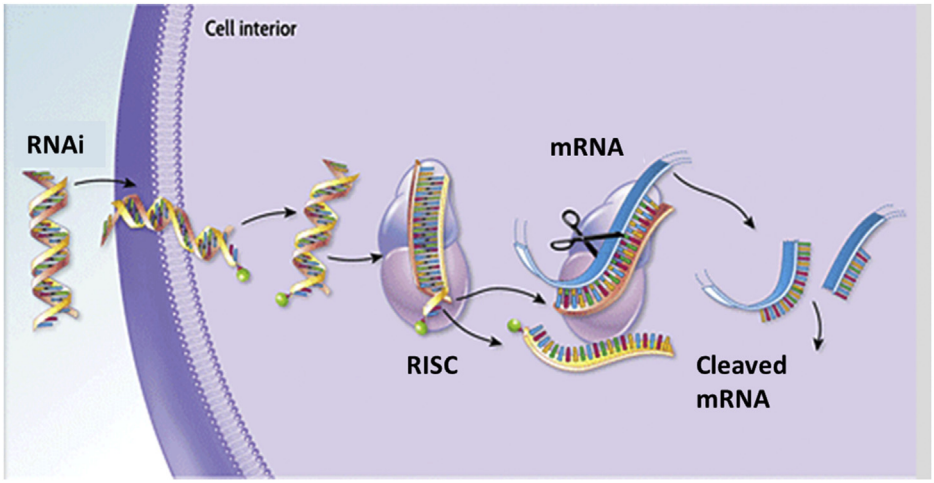

Fig. 1. Therapeutic Potential of RNAi. RNAi enters cells by various methods. Recent modifications to delivery including use of lipid nanoparticles and conjugation to *N*-acetyl galactosamine have allowed for the possibility of treatment of liver specific diseases. Once in the cytoplasm, RNAi binds to target mRNA resulting in decreased target protein expression.

simulating chylomicron remnants following intravenous injection. Conjugation to GalNAc uses specific receptors on hepatocytes and enables subcutaneous injection [5–7]. These recent advances in liver-targeting RNAi therapeutics have provided an opportunity to reduce endogenous oxalate synthesis and thus reduce calcium oxalate kidney stone risk [8–10].

2. Oxalate synthesis

Endogenous synthesis of oxalate occurs primarily in the liver and contributes to calcium oxalate kidney stone disease. In the rare Primary Hyperoxaluria (PH) diseases, an increased endogenous oxalate synthesis can lead to recurrent calcium oxalate kidney stone formation, and in some cases to end stage renal disease. Some of the more severe cases necessitate combined liver-kidney transplant to treat the disease which requires life-long immune suppression and carries significant mortality risk. The pathways to oxalate synthesis, the locations of genetic defects resulting in PH, and potential targets for RNAi can be seen in Fig. 2. There are 3 known forms of PH. Primary hyperoxaluria type 1 (PH1), an inherited rare disease of glyoxylate metabolism, arises from mutations in the enzyme alanine-glyoxylate aminotransferase. The resulting deficiency in this enzyme leads to the inability to convert glyoxylate to glycine and the resulting buildup of glyoxylate leads to increased oxalate production. Primary hyperoxaluria type 2 involves a mutation in glyoxylate reductase (GHPR) which prevents the conversion of glyoxylate to glycolate. The disease manifestations in PH2 are usually less severe than PH1. PH type 3 results from a deficiency in an aldolase that normally cleaves 4-hydroxy-2-oxyglutarate (HOG), a metabolite of hydroxyproline, into pyruvate and glyoxylate. Despite the knowledge of metabolic pathways in primary hyperoxaluria, treatment options are limited; a small subset of PH1 patients are able to manage this disease with vitamin B6 treatments [11,12].

3. Current status

Recently, there have been reports of the possible therapeutic benefit of RNAi therapeutics in the treatment of primary hyperoxaluria. Fig. 2 illustrates the endogenous oxalate pathways and the potential RNAi targets, glycolate oxidase (GO) and hyroxyproline dehydrogenase (HYPDH). These targets appear to be safe as individuals with mutations in these genes have no adverse phenotypic presentations [13–15]. The development of investigational RNAi therapeutics (by Alnylam Pharmaceuticals and Discerna Pharmaceuticals) targeting liver GO have been shown to deplete oxalate production. Liebow et al. demonstrated subcutaneous administration of ALN-GO1 resulted in potent, dose-dependent, and durable silencing of the mRNA encoding GO in wild-type mice, rats, and nonhuman primates [9]. Notably, ALN-GO1 reduced urinary oxalate concentration up to 50% after a single dose in the genetic mouse model of PH1, and up to 98% after multiple doses in a rat model of hyperoxaluria. In a phase I/II study, 32 healthy volunteers were treated with ALN-GO1 with a good safety profile (http://investors.alnylam.com/releasedetail.cfm?ReleaseID=990764). PH1 patients are now being recruited to determine if urinary oxalate levels can be lowered. Dutta et al. used a different RNAi construct targeting GO. The construct was a dicer substrate RNAi in lipid nanoparticles that was delivered intravenously into mice and nonhuman primates. The data demonstrated reduction of hepatic GO and normalization of urinary oxalate and reduction of CaOx deposition in the mouse model of PH1 [8].

The enzyme, HYPDH, involved in hydroxyproline catabolism is another RNAi target. Hydroxyproline is thought to contribute ≥25% of endogenous oxalate [16,17]. RNAi targeting liver HYPDH demonstrated decreased oxalate excretion in a mouse model of PH1 and the wild-type mouse fed a low oxalate diet [10]. HYPDH is present in other organs, including the kidney, and thus improvements in RNAi delivery to multiple organs is needed to maximize the reduction of oxalate by RNAi targeting HYPDH [18].

4. Future directions

Two companies, Alnylam and Dicerna Pharmaceuticals, have developed a pipeline of RNAi therapeutics for treating PH1 by targeting GO. The leading candidates are currently in early phase clinical trials and have shown good tolerability and safety as well as proof of concept in normal subjects. The future direction of RNAi therapeutics in kidney stone disease will be dependent on the success of the current clinical trials treating PH1 patients. Recent closure in October 2016 of an advanced phase III RNAi trial for a subset of patients with hereditary amyloidosis for safety concerns has led to consternations about RNAi's future (http://investors.alnylam.com/releasedetail.cfm?ReleaseID=992320).