Using RNAi in the treatment of cardiovascular diseases - therapeutics based on siRNA overview

Barbara Rusinowska

Student Research Group at the Chair and Department of Epidemiology and Clinical Research Methodology; Medical University of Lublin

Contact: rusinowskabarbara4@gmail.com; +48 794303542
Orcid: 0000-0002-8207-1042

Abstract

RNA interference (RNAi) discovered in the 1990s by Fire and Mello plays a role in silencing gene function. One type of RNAi is siRNA, which is a double-stranded molecule of 20-25 base pairs. This molecule is made by cleaving double-stranded RNA by the enzyme Dicer. siRNA binds to the protein complex with ribonuclease activity - RISC (RNA-induced silencing complex). The resulting complex binds to the mRNA and cuts it into parts, which blocks the formation of the protein encoded by the mRNA. This property has been exploited in the production of siRNA-based drugs. However, the instability of siRNA molecules turned out to be difficult - the challenge was to properly modify the structure of siRNA in order to increase the stability and half-life and select the appropriate method of delivering the molecule to the body. Many siRNA-based drugs have already been developed, but most are in clinical trials. In this review, we present the role of RNA interference in therapeutics production and use of siRNA in cardiovascular diseases treatment.

Keywords: RNAi; RNA interference; siRNA; RNA; therapeutics; cardiovascular diseases

Introduction

Interest in the use of mRNA dates back to the 1960s, but the main obstacles at that time were instability and immunogenicity of mRNA. Only the modification of mRNA by introducing modified nucleosides into its sequence (modification of uracil ribose in order to produce the so-called pseudouracil undetectable by the immune system) and the development of new methods of packaging and delivering messenger-RNA (recent years) resulted in the fact that it began to be seen as a huge potential - not only by highly efficient transfection, but also by extending the time of protein expression [1]. Further research on RNA molecule led to the discovery of phenomenon of the Interference RNA – RNAi [2,3].

In this review, we summarize the role of RNA interference (siRNA and miRNA) in therapeutics production and use of siRNA in cardiovascular diseases treatment.

The potential of RNA molecule

Messenger-RNA, apart from the above-mentioned features, has numerous advantages - independence from the cell nucleus (avoiding the transcription process which takes place in the cell nucleus, where the DNA is rewritten into mRNA – done mRNA is given, it makes the material is ready for the translation process taking place in the cytoplasm, resulting in expression proteins - here mRNA has a decisive advantage over DNA due to a much higher efficiency), the inability to penetrate the host genome and thus limiting the insertion mutation, ease of synthesis due to DNA transcription (which in addition is a fast and inexpensive process) and the ability to express any proteins (the possibility of controllability of protein synthesis in cells; here a huge potential for the treatment of the vast majority of diseases - through the production of properly functioning proteins, as well as antigens that induce the production of antibodies by the organism) [4,5].

RNAi (RNA interference), which has been researched since the late 1990s by Fire and Mello (1998), act a significant role in silencing certain genes [3,6]. There are two main types of RNAi - siRNA (double-stranded, small interfering RNAs that bind and degrade defined mRNAs; consist of 20-25 nucleotides in lenght) and miRNAs (single-stranded but less homology to mRNA than in the case of siRNA; consist of 17-25 nucleotides in lenght). Both types target mRNAs using base-pair recognition and initiate mRNA degradation and then decreases the levels of the corresponding protein. Although the two molecules are similar, there are some differences. After transfection, siRNA is split by helicase (ATP-dependent), the non-degraded antisense strand is incorporated into the RISC interference complex (activation of the complex), this complex then attaches to the complementary siRNA messenger-RNA and cuts it (degradation of mRNA) and block the production of a specific protein. A similar mechanism involving the RISC complex is represented by miRNA, which apart from degradation causes very high specificic inhibition of translation [3,6,7,8,9,10]. The miRNA molecule generally bind to the untranslated region 3′UTR of mRNAs and then repress their translation or recruit deadenylases and/or decapping enzymes to simplify the degradation of specific mRNA [8]. The main challenge in using RNAi-based therapies has been to find the ideal method of delivering such material to cells. Numerous studies have considered direct transmission by injection, but also systemic transmission using nanoparticles, liposomes and aptamers (although the target was a limitation here - this method may only be suitable for specific organs and body compartments).

The most common are RNAi delivery by lipid carrier transfection (especially using cationic lipid-based reagents) and viral transduction (usually adenoviruses and letinoviruses are used as vector) [9,11]. The half-life of siRNA and miRNA can be extended due to chemical modification (2'-O-methyl substitution for ribose-2' and modification of phosphorothioate bonds). Extending the half-life has very little effect on gene silencing. Modifications can also be related to the blockade of the nucleic acid (greater hybridization properties). Increasing the stability of siRNA molecules can be done by replacing the unstable and highly charged phosphodiester backbone with a phosphorothioate (PS) backbone. Resistance to the action of nuclease and phosphodiesterase and at the same time increasing the hydrophobicity of molecules (more beneficial pharmacokinetics) is carried out by replacing one of the non-bridge oxygen atoms on the phosphate group with a sulfur atom. This modification is considered to be one of the most important and successful modifications in this field but some studies show that the modified molecule may have a reduced ability to silence genes and thus be less effective (possibly this is by interfering with RISC activation due to the presence of PS at the center of the strand). Research results suggest that partially modified molecules remain effective and PS modifications at the end of the strand are more tolerated [12,13,14]. Thanks to carriers in the form of liposomes (here the possibility of penetrating the cell membrane, because of the ability to diffusion and endosomal penetration through the cell wall), nanoparticles or aptamers (most often are used oligonucleic acids and peptide molecules), it is possible for molecules to recognize specific receptors on cells, which gives high efficiency in targeted therapies [15].

Overview of siRNA-based drugs

Inclisiran is a drug made by Novartis Europharm used to lower LDL levels in adult patients with primary hypercholesterolaemia (heterozygous familial hypercholesterolaemia and polygenic hypercholesterolaemia) and mixed dyslipidaemia, which was approved in the EU in December 2020. This drug uses a long-acting, synthetic siRNA molecule that has undergone numerous modifications to increase stability, which is directed against the PCSK9 receptors in hepatocytes responsible for the metabolism of low-density lipoproteins, which allows for an effective reduction of LDL concentration in the blood, which is the most effective mechanism of drugs so far. Lipid-lowering drugs, especially in patients at risk of coronary heart disease, and it is the first drug with such a mechanism using siRNA [16,17,18]. Inclisiran consists of two threads - the guide thread and the passenger thread, of which the 3 'end of the passenger thread is coupled with three-antennae N-acetylgalactosamine (GalNAc) carbohydrates, which abundantly bind asialoglycoprotein receptors (ASGPR) in the liver, leading to targeted drug uptake by hepatocytes. The guide strand, connecting with the RISC complex, causes binding of PCSK9 mRNA and its degradation, preventing the production of the PCSK9 protein. Inclisiran is most often used in addition to a low-fat diet and in combination with statins, although in patients with statin intolerance, inclisiran can be used alone or in combination with other lipid-lowering drugs [19,20]. Inclisiran is available as pre-filled syringes as a solution for subcutaneous injection - each pre-filled syringe contains 284 mg of inclisiran in 1.5 ml of solution. The unquestionable advantage of inclisiran is the low frequency of administration of this drug - the currently recommended dose is 284 mg of the drug administered consecutively on day 1, 90, and then every 6 months.

The EU approval of the drug was based on the results of the ORION clinical trials, which confirmed the efficacy and safety of Inclisiran in lowering LDL levels in patients with hypercholesterolaemia at the maximum doses of statins. Inclisiran lowers LDL levels by more than 50% with one dose every 6 months, which is a great innovation in the treatment of hyperlipidemia, where statins alone reduce LDL by 10-20%, and inclisiran is well tolerated by patients [21].

Patisiran is a hepatically directed therapeutic used in the treatment of hereditary transthyretin amyloidosis with polyneuropathy and transthyretin amyloidosis with cardiomyopathy. This drug use the interference process to reduce the synthesis of mutant and wild-type transthyretin (by targeting the 3′UTR of transthyretin mRNA). Patisiran is available as a 10 mg/5 mL lipid complex injection administrated in intravenous infusion over 80 minutes. FDA recommendation of dosage of patisiran is 0.3 mg/kg every 3 weeks. APOLLO studies on patisiran in people with transthyretin amyloidosis with cardiomyopathy (3rd phase of trials) showed decreasion of mean left ventricular wall thickness, left ventricular global longitudinal strain and NT-proBNP level in comparison with placebo [14,22,23].

Vutisiran is also used in the treatment of hereditary transthyretin amyloidosis with polyneuropathy and transthyretin amyloidosis with cardiomyopathy and it also targets liver cells. This drug consists of two strands, a 21-base strand and a 23-base strand, and is coupled to GalNAc, which enables specific and high uptake by hepatocytes. The HELIOS-A trials compared the effectiveness of vutisiran and patisiran in the treatment of hereditary transthyretin amyloidosis with polyneuropathy. Results was published in January 2022 and showed a decrease TTR levels as well as a decrease the heart failure marker NT-proBNP. The FDA is currently considering the introduction of vutisiran to treat hereditary ATTR polyneuropathy. The HELIOS-B study, which is scheduled to end in 2024, aims to evaluate the effectiveness of vutrisiran in hATTR patients with cardiomyopathy compared to placebo. The drug is administered as a subcutaneous injection once every three months, which gives it an advantage over patisiran [14,24]

Givosiran is subcutaneous siRNA drug approved by the FDA on November 20, 2019, used in patients with acute hepatic porphyria, a genetic disorder associated with a mutation in the heme synthesis pathway and induction of the first enzyme in this pathway - aminolevulin synthase 1 (ALAS1) but also associated with chronic comorbidities, including hypertension. It causes an increase in the neurotoxic metabolites of aminolevulinic acid (ALA) and porphobilinogen (PBG), leading to a life threatening condition. The goal of the drug is to bind to a target sequence on the mRNA intended for ALAS1, which reduces the synthesis of this enzyme and thus the toxic metabolites of the pathway (and also reducing frequency of attacks of the disease). Givosiran consists of a 21-base sense strand and a 23-base antisense strand and is conjugated to tri-GalNAc allowing enhanced uptake by hepatic cells. Despite its effectiveness, the drug has a number of side effects with prolonged use, including iron overload of the liver, thrombocytopenia, phlebitis and tachyphylaxis. Due to a transient increase in creatinine, a negative effect on the kidneys and the progression of their disease in the case of long-term use of givosiran cannot be excluded. The recommended dose is 189 mg / ml and dose of 2.5 mg / kg once a month. [14,25,26].

Fitusiran is an siRNA drug developed for the treatment of haemophilia A and B directed against antithrombin. By inhibiting the production of anticoagulants, it reduces the number of bleeding, which is a promising method because the effect of the application is the same as with the use of the missing coagulation factors, but it is not burdened with the risk of autoantibody formation, and it is not as onerous. The drug works by binding to antithrombin mRNA, which increases the level of thrombin. Fitusiran consists of a 21-base strand and a second - 23-base strand and is conjugated to tri-GalNAc leading to the liver where antithrombin is synthesized. The results of clinical trials are promising - decreased levels of antithrombin and decreased bleeding frequency were observed in patients with haemophilia A and B. Adverse events were rare and the most common were ALT increases and injection site erythema [14,27].

Teprasiran is an intravenous drug consists of two 19-base strands with 19 2'-OMe substituted bases, designed for the prophylactic treatment of acute kidney injury (AKI) following kidney transplantation or cardiovascular surgery in high-risk patient. Teprasiran works by binding to p53 mRNA molecules (a key mediator of AKI) majorly to the proximal tubule brush border. The teprasiran phosphate backbone is not modified and is administered as a naked siRNA molecule without a specific delivery system or conjugated targeting ligand, allowing the drug to be rapidly excreted by the kidneys and reabsorbed by the proximal tubular cells (PTC), where it accumulates. Clinical trials have demonstrated the safety and tolerability of teprasiran in patients after cardiac operations. In addition, teprasiran reduced the incidence of AKI after the interventions as well as its duration and severity [14,28].

Conclusion

Presented features cause that RNA is becoming more and more approved and can be used in the pharmaceutical industry for the production of drugs, which in the long run may bring great progress in the development of medicine. Many of the drugs presented in this review require further research on their effectiveness, but it seems that siRNA-based drugs may become the future of medicine and allow patients to more conveniently treat of cardiovascular diseases (inclisiran, patisiran, vutisiran, givosiran, fitusiran) and prevent some complications after cardiac procedures (teprasiran).

References:
1. Liang X, Li D, Leng S, Zhu X. RNA-based pharmacotherapy for tumors: From bench to clinic and back. Biomed Pharmacother. 2020 May;125:109997. doi: 10.1016/j.biopha.2020.109997. Epub 2020 Feb 13. PMID: 32062550.
2. Wilson RC, Doudna JA. Molecular mechanisms of RNA interference. Annu Rev Biophys. 2013;42:217-39. doi: 10.1146/annurev-biophys-083012-130404. PMID: 23654304; PMCID: PMC5895182.
3. Caillaud M, El Madani M, Massaad-Massade L. Small interfering RNA from the lab discovery to patients' recovery. J Control Release. 2020 May 10;321:616-628. doi: 10.1016/j.jconrel.2020.02.032. Epub 2020 Feb 19. PMID: 32087301.
4. Weng Y, Li C, Yang T, Hu B, Zhang M, Guo S, Xiao H, Liang XJ, Huang Y. The challenge and prospect of mRNA therapeutics landscape. Biotechnol Adv. 2020 May-Jun;40:107534. doi: 10.1016/j.biotechadv.2020.107534. Epub 2020 Feb 21. PMID: 32088327.

5. Dong Y, Siegwart DJ, Anderson DG. Strategies, design, and chemistry in siRNA delivery systems. Adv Drug Deliv Rev. 2019 Apr;144:133-147. doi: 10.1016/j.addr.2019.05.004. Epub 2019 May 15. PMID: 31102606; PMCID: PMC6745264.

6. Speiser JJ, Erşahin C, Osipo C. The functional role of Notch signaling in triple-negative breast cancer. Vitam Horm. 2013;93:277-306. doi: 10.1016/B978-0-12-416673-8.00013-7. PMID: 23810012.

7. Lee LW, Zhang S, Etheridge A, Ma L, Martin D, Galas D, Wang K. Complexity of the microRNA repertoire revealed by next-generation sequencing. RNA. 2010 Nov;16(11):2170-80. doi: 10.1261/rna.2225110. Epub 2010 Sep 28. PMID: 20876832; PMCID: PMC2957056.

8. Bejar N, Tat TT, Kiss DL. RNA Therapeutics: the Next Generation of Drugs for Cardiovascular Diseases. Curr Atheroscler Rep. 2022 May;24(5):307-321. doi: 10.1007/s11883-022-01007-9. Epub 2022 Apr 2. PMID: 35364795; PMCID: PMC8975710.

9. Langer C, Rücker FG, Buske C, Döhner H, Kuchenbauer F. Targeted therapies through microRNAs: pulp or fiction? Ther Adv Hematol. 2012 Apr;3(2):97-104. doi: 10.1177/2040620711432582. PMID: 23556116; PMCID: PMC3573433.

10. Zhao Z, Lin CY, Cheng K. siRNA- and miRNA-based therapeutics for liver fibrosis. Transl Res. 2019 Dec;214:17-29. doi: 10.1016/j.trsl.2019.07.007. Epub 2019 Aug 13. PMID: 31476281; PMCID: PMC6848786.

11. Thermo Fisher Scientific. Delivering RNAi to Cells - Transfection and Viral Delivery. https://www.thermofisher.com/pl/en/home/life-science/rnai/delivering-rnai-to-cells-transfection-and-viral-delivery.html.html (14.07.2022)

12. Dowdy SF. Overcoming cellular barriers for RNA therapeutics. Nat Biotechnol. 2017 Mar;35(3):222-229. doi: 10.1038/nbt.3802. Epub 2017 Feb 27. PMID: 28244992.

13. Sun Y, Zhao Y, Zhao X, Lee RJ, Teng L, Zhou C. Enhancing the Therapeutic Delivery of Oligonucleotides by Chemical Modification and Nanoparticle Encapsulation. Molecules. 2017 Oct 13;22(10):1724. doi: 10.3390/molecules22101724. PMID: 29027965; PMCID: PMC6158866.

14. Zhang MM, Bahal R, Rasmussen TP, Manautou JE, Zhong XB. The growth of siRNA-based therapeutics: Updated clinical studies. Biochem Pharmacol. 2021 Jul;189:114432. doi: 10.1016/j.bcp.2021.114432. Epub 2021 Jan 26. PMID: 33513339; PMCID: PMC8187268.

15. Chakraborty C, Sharma AR, Sharma G, Doss CGP, Lee SS. Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. Mol Ther Nucleic Acids. 2017 Sep 15;8:132-143. doi: 10.1016/j.omtn.2017.06.005. Epub 2017 Jun 12. PMID: 28918016; PMCID: PMC5496203.

16. Latimer J, Batty JA, Neely RD, Kunadian V. PCSK9 inhibitors in the prevention of cardiovascular disease. J Thromb Thrombolysis. 2016 Oct;42(3):405-19. doi: 10.1007/s11239-016-1364-1. Erratum in: J Thromb Thrombolysis. 2016 Oct;42(3):420. PMID: 27095708; PMCID: PMC5010583.

17. Dyrbuś K, Gąsior M, Penson P, Ray KK, Banach M. Inclisiran-New hope in the management of lipid disorders? J Clin Lipidol. 2020 Jan-Feb;14(1):16-27. doi: 10.1016/j.jacl.2019.11.001. Epub 2019 Nov 12. PMID: 31879073.

18. Kosmas CE, Muñoz Estrella A, Sourlas A, Silverio D, Hilario E, Montan PD, Guzman E. Inclisiran: A New Promising Agent in the Management of Hypercholesterolemia. Diseases. 2018 Jul 13;6(3):63. doi: 10.3390/diseases6030063. PMID: 30011788; PMCID: PMC6163360.

19. Debacker AJ, Voutila J, Catley M, Blakey D, Habib N. Delivery of Oligonucleotides to the Liver with GalNAc: From Research to Registered Therapeutic Drug. Mol Ther. 2020 Aug 5;28(8):1759-1771. doi: 10.1016/j.ymthe.2020.06.015. Epub 2020 Jun 17. PMID: 32592692; PMCID: PMC7403466.

20. Lamb YN. Inclisiran: First Approval. Drugs. 2021 Feb;81(3):389-395. doi: 10.1007/s40265-021-01473-6. Erratum in: Drugs. 2021 Jun;81(9):1129. PMID: 33620677; PMCID: PMC7900795.

21. Rogula S, Błażejowska E, Gąsecka A, Szarpak Ł, Jaguszewski MJ, Mazurek T, Filipiak KJ. Inclisiran-Silencing the Cholesterol, Speaking up the Prognosis. J Clin Med. 2021 Jun 2;10(11):2467. doi: 10.3390/jcm10112467. PMID: 34199468; PMCID: PMC8199585.

22. Adams D, Gonzalez-Duarte A, O'Riordan WD, Yang CC, Ueda M, Kristen AV, Tournev I, Schmidt HH, Coelho T, Berk JL, Lin KP, Vita G, Attarian S, Planté-Bordeneuve V, Mezei MM, Campistol JM, Buades J, Brannagan TH 3rd, Kim BJ, Oh J, Parman Y, Sekijima Y, Hawkins PN, Solomon SD, Polydefkis M, Dyck PJ, Gandhi PJ, Goyal S, Chen J, Strahs AL, Nochur SV, Sweetser MT, Garg PP, Vaishnaw AK, Gollob JA, Suhr OB. Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. N Engl J Med. 2018 Jul 5;379(1):11-21. doi: 10.1056/NEJMoa1716153. PMID: 29972753.

23. Clinical Trials. APOLLO-B: A Study to Evaluate Patisiran in Participants With Transthyretin Amyloidosis With Cardiomyopathy (ATTR Amyloidosis With Cardiomyopathy) https://www.clinicaltrials.gov/ct2/show/NCT03997383 (16.07.2022)

24. Clinical Trials Arena. Vutrisiran HELIOS-A results bolster its FDA application https://www.clinicaltrialsarena.com/comment/vutrisiran-results-fda-application/ (16.07.2022)

25. Majeed CN, Ma CD, Xiao T, Rudnick S, Bonkovsky HL. Spotlight on Givosiran as a Treatment Option for Adults with Acute Hepatic Porphyria: Design, Development, and Place in Therapy. Drug Des Devel Ther. 2022 Jun 16;16:1827-1845. doi: 10.2147/DDDT.S281631. PMID: 35734365; PMCID: PMC9208469.

26. Lazareth H, Poli A, Bignon Y, Mirmiran A, Rabant M, Cohen R, Schmitt C, Puy H, Karras A, Gouya L, Pallet N. Renal Function Decline With Small Interfering RNA Silencing Aminolevulinic Acid Synthase 1 (ALAS1). Kidney Int Rep. 2021 Apr 15;6(7):1904-1911. doi: 10.1016/j.ekir.2021.04.004. PMID: 34307985; PMCID: PMC8258458.

27. Pasi KJ, Lissitchkov T, Mamonov V, Mant T, Timofeeva M, Bagot C, Chowdary P, Georgiev P, Gercheva-Kyuchukova L, Madigan K, Van Nguyen H, Yu Q, Mei B, Benson CC, Ragni MV. Targeting of antithrombin in hemophilia A or B with investigational siRNA therapeutic fitusiran-Results of the phase 1 inhibitor cohort. J Thromb Haemost. 2021 Jun;19(6):1436-1446. doi: 10.1111/jth.15270. Epub 2021 May 18. PMID: 33587824; PMCID: PMC8251589.

28. Thielmann M, Corteville D, Szabo G, Swaminathan M, Lamy A, Lehner LJ, Brown CD, Noiseux N, Atta MG, Squiers EC, Erlich S, Rothenstein D, Molitoris B, Mazer CD. Teprasiran, a Small Interfering RNA, for the Prevention of Acute Kidney Injury in High-Risk Patients Undergoing Cardiac Surgery: A Randomized Clinical Study. Circulation. 2021 Oct 5;144(14):1133-1144. doi: 10.1161/CIRCULATIONAHA.120.053029. Epub 2021 Sep 3. PMID: 34474590; PMCID: PMC8487715.