Review Article

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RNA DRUG AS THERAPEUTIC AGENTS: A REVIEW BASED ON LITERATURE.

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ABSTRACT

Small RNA-targeted drugs have received significant interest from the public and researchers in the last decade due to their capability to treat different illnesses. RNA drugs correspond to the small molecules that interact with and regulate the expression of particular genes at the RNA level. Unlike small molecular chemicals, RNA drugs act directly on the nucleus and gene to produce efficient and effective curative functions. Some RNA drugs are siRNA, ASOs, and mRNA, although the last one is used in vaccines. Both compounds, SiRNA and ASOs, act by attaching to the target mRNA chain and, as a result, the latter can be degraded or prevented from being translated into a protein.On the other hand, mRNA vaccines involve introducing a particular mRNA sequence into cells, and the outcome is that cells synthesize a specific protein that can be a viral antigen to spark an immune response. The RNA drugs have demonstrated significant outcomes with initial trials for diseases like cancer, genetic disorders, and infections. For instance, siRNA-based drugs are introduced for the treatment of hereditary transthyretin amyloidosis, which is a rare genetic disease and ASO-based drugs for spinal muscular atrophy. mRNA vaccines have also been developed to fight against the epidemic such as COVID-19. However, a few significant issues have to be considered about RNA drugs. These are problems of drug delivery and stability and have to do with side or off-target effects. Despite these hurdles, RNA drugs are a promising new disease category that this technique can treat. As the research work in RNA medicine grows, the public might observe more RNA-based drugs being approved for clinical use.

Keywords: RNA drugs, siRNA, ASOs, mRNA vaccines, gene therapy.

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INTRODUCTION

RNA therapeutics A substantial number of studies conducted over the last couple of decades have identified RNAs as drug targets, and this consequently has contributed to our understanding that RNA-based drugs will be effective in treating a multitude of disease conditions. RNA drugs target the genetic material that encodes proteins instead of protein-based traditional small molecule drugs designed based on known structure-function gradients. siRNA and mRNA are the two primary classes of RNA drugs. Functionally, siRNA binds to and destructs the matching messenger RNA (mRNA) type and is encoded for a specific protein. Instead, the mRNA provides cells with directions for creating particular proteins that may help address diseases caused by protein shortages. RNA drugs have immense performance in the treatment of various diseases like cancer, virgin disorders and viral infection. The US Food and Drug Administration (FDA) approved the first RNA drug for Onpattro in 2018, which treats a rare genetic disorder called hereditary transthyretin-mediated amyloidosis. Since then, many more RNA drugs have been approved or are being studied in clinical trials, including mRNA vaccines for COVID-19. RNA drugs come with great expectations and new challenges related to their development and delivery. RNA is a fragile molecule that can be easily broken down in the body. However, delivering the protein to the appropriate cells or tissues is often challenging. Furthermore, RNA drugs may also trigger off-target effects and immune responses in a subset of the patients. Among these, the promise of RNA drugs to transform the treatment options for multiple diseases is spurring intensive research and development in this space. It seems likely that, as far as we know, RNA biology and the features supporting its druggable expansion, but this class will still grow significantly in its reach within medicine.

Ancient and Modern RNA Therapeutics Strategies:

RNA therapeutics have come a long way since then, and technological advances and new methods all contribute to developing RNA drugs as safer, more efficacious tools. This answer will explain traditional and new methods utilized in developing RNA drugs, fleshing them out further.

Applications of Old Methods for Development Of RNA-Based Drugs:

RNA Program Development initially targets identifying, designing, and synthesis of RNA molecules. Antisense oligonucleotides (ASOs), designed short, synthetic RNA molecules that bind to messenger RNA(mRNA) and block the production of disease-causing proteins, were an initial class of therapeutic agent developed. ASOs were initially made by chemically synthesizing them, which took place in multiple steps as each nucleotide building block was added one at a time to obtain the desired sequence. It was a long, complex operation that required time and effort by the pound to give low or poor yield-sensitive pure products. In vitro transcription (IVT) was an alternative method of RNA synthesis in which large amounts of RNA were produced from a DNA template by synthesizing enzymes, such as the ones found on T7 and SP6 bacteriophages. IVT is a straightforward, scalable approach, but the resulting RNA can be impure and trailed by undesired contaminants that impact their efficacy and safety. The Next Challenge: Drug Delivery Drug delivery was one of the biggest.

Hurdles when it came to RNA drug development. Previously, RNA molecules have been delivered using cationic lipids/polymers, which were shown to be toxic and immunostimulatory.

Novel Strategiesfor RNA Drug Discovery:

The rapid development of RNA synthesis, modification, and delivery technologies has created new opportunities to discover effective RNA drugs.

RNA Synthesis:

For example, solid-phase synthesis is a method in which the RNA molecule is chemically synthesized on a column using an automated synthesizer. It is a high-yield, top-quality RNA molecule synthesis method.

RNAModification:

Since RNA molecules have an inherently short half-life, chemical modifications to these RNAs can also improve product stability and possibly efficacy. One such change is replacing units with locked nucleic acids (LNAs) to modify human RNA for better binding and a more specific mRNA target.

Delivery:

In conclusion, and perhaps most importantly, new cutting-edge delivery systems have been developed as part of the latest breakthroughs in RNA drug delivery. One of these is using lipid nanoparticles, which serve as a vehicle that stabilizes RNA molecules from degradation and helps ferry them into the cells you are trying to target. Lipid nanoparticles are highly versatile, readily manufactured, and have demonstrated efficacy in both preclinical animal models and humans in clinical trials.

In conclusion, the history of RNA drug development is a story of advancement as new and improved formulations for synthesis, modification & delivery have shaped this field over time. These advances enhance the accuracy and safety profile of RNA-based medicines, making them highly promising for treating various diseases.

RNADrug Modifications

RNA drugs, also called nucleic acid therapeutics, target the RNA of cells to address different diseases]. These drugs can be developed to silence or tone up the expression of specific genes. Many classes of structural modifications can be introduced in RNA drugs to enhance their efficacy, stability and specificity. Notably, (position of redeliver), an ethoxy acetal group was developed (~590) to enable a visible "kink" when in the conformation found in RNA and ~45% higher basicity at greater pH values than Ph; these modifications are also less luminescent with colloidal binding [64].2'-O-methyl modification adds a methyl group at the 2' position of ribose sugar in an RNA molecule. This alteration improves RNA drugs' stability and target specificity while protecting them from nuclease degradation. 2′-OMe-modified RNA is less immunogenic as well.

Phosphorothioate Chemical Substitution:

Phosphorothioate modification replaces one non-bridging oxygen atom in the PO4 inter nucleic linkage within an RNA molecule; phosphorous must be linked with sulfur. This modification provides excellent stability and reduces the ability of the RNA drug to be digested by nucleases. Phosphorothioate modification enhances cellular uptake and tissue distribution of RNA.

Locked Nucleic Acid (LNA) Modification

A type of chemical modification to RNA is locked nucleic acid (LNA), where LNA replaces some nucleotides during synthesis. LNA nucleotides contain a methylene bridge connecting the 2' oxygen and 4' carbon atoms, which confines the sugar ring in an opposite predetermined conformation. These modifications also make the RNA drug more stable, allow it to be specific, and increase its affinity for its target.

Peptide Nucleic Acid (PNA)modification:

PNAs are a type of PNA oligomer that replaces the conventional RNA sugar-phosphate backbone with special peptide-like units. By introducing this modification, the RNA drug's stability, specificity, and binding affinity to its target are enhanced, along with improved resistance to nuclease degradation. PNA-modified RNA is also not likely to activate an immune response.

Methylation of 5-methylcytosine (5-mC)

This involves adding a methyl group to the five position in the cytosine base within an RNA molecule and is referred to as 5-mC modification. These changes provide more excellent stability and specificity for the RNA drug while increasing resistance to nuclease degradation. Moreover, 5- mC-modified RNA is more readily taken up by cells in vivo and also displays improved tissue distribution.

Unnatural Base Pair (UBP)

Modifying unnatural base pairs by insertion into transcribed RNA Synthetic nucleotides that can base pair with one another and naturally occurring nucleotides, thus opening up possibilities for synthesizing RNAs possessing unique properties. The modification can stabilize the structure and enhance specificity or affinity,

Allowing it to perform new functions. Therefore, these changes, in addition to increasing potency, can combine to provide improved stability and specificity of RNA drugs, making them essential tools for treating different diseases.

RNA Drug Delivery Methods

Chemical Approaches to RNA Delivery for Therapeutic Mechanisms RNA drug delivery is a fast-growing area, and many chemical strategies have been developed to transport RNA therapeutics into cells or tissues. Two major groups of RNA molecules are being used in the field: siRNA and mRNA.

Chemical methods in RNA drug delivery Lipid-based delivery systems:

These use cationic lipids to complex with the negatively charged RNA molecules [180], aiding cellular uptake. Designing these delivery systems can improve RNA stability, cell type targeting and nuclease protection.

Polymersome Delivery systems

These include encapsulating RNAs within biodegradable or non-biodegradable polymers, enabling protection from degradation. Moreover, such delivery systems can be engineered to deliver RNAs that target specific types of cells and release RNA molecules under regulated conditions.

Delivery systems based on peptides

They include cationic peptides that can bind reversibly with RNA molecules, resulting in complex formation and increased biological uptake. Furthermore, other peptide-based delivery systems can be formulated to increase RNA stability and cell-specific targeting.

Delivery systems based on aptamers:

These included using aptamers, which are small strands of either RNA or DNA that can bind to targets. The structurally diverse nature of various aptamers allows for the possibility of using their specificity in targeting particular cell types or tissues by selecting appropriate aptamers that strongly bind to specific receptors located on cellular surfaces, membrane proteins and other biomolecules.

Inorganic nanoparticles:

For example, the combination of nanoparticles and RNA encapsulation for cell uptake (materials here could be gold or silica as well as iron oxide) is being tested. Inorganic nanoparticles can also be functionalized with ligands to target specific cell types or tissues. The delivery of RNA can also be achieved by physical methods, such as electroporation using an electric field to transiently disrupt cell membranes and encourage the absorption of R-NA molecules. Other physical methods are used, such as ultrasound-mediated delivery and microinjection. All three of these approaches have their merits and limitations, with the choice of the delivery method being dependent on the RNA therapeutic intended for delivery and the cell/tissue targets/therapeutic effect required. These are the critical points to be considered in successfully delivering RNA drugs, along with a given delivery system's safety and efficacy parameters.

Physical methods of RNA drug delivery

RNA therapeutics are potential compounds for treating many diseases, such as cancer, viral infection and genetic disorders. Nevertheless, RNA drugs are volatile and have shown poor cell penetration, the most significant issue once delivered to target cells. Physical delivery methods have been developed to cope with these, and in this answer, I will discuss some of the most widely used physical delivery approaches for RNA drugs.

Electroporation:

It is a method where one applies short electric pulses to open the cell membrane and let RNA molecules in. In this manner, the target is suspended in an RNA solution and subsequently used with an electric current. The electrical field transiently punches holes in the cell membrane, thus opening paths for RNA molecules to enter each cell. Among the other mechanisms used to deliver RNA drugs, electroporation is particularly useful for in vitro and ex vivo applications (to treat cells outside of a patient), such as gene editing with stem cells.

Microinjection:

Thus, microinjection enables the blunt transfer of RNA molecules through a microneedle directly into the cytoplasm of target cells. This procedure is very accurate and can deliver many RNA molecules to individual cells or tissues. However, this technique is invasive and requires specialized equipment and corresponding expertise.

Ultrasound:

Thus, we developed ultrasound to penetrate the cell membrane and improve RNA uptake by target cells. RNA is mixed with microbubbles in this technique and then injected into the bloodstream. During the treatment, ultrasound waves act upon this tissue and cause the microbubbles to oscillate, breaking the cell membrane and allowing RNA molecules to enter the cells. First, ultrasound is noninvasive and can be applied in vitro (3), making it a very appealing therapeutic framework.

Magnetofection:

Magnetofection: Here, magnetic fields are applied to boost the penetration of RNA molecules into target cells. This method involves mixing RNA molecules with magnetic nanoparticles and addition to target cells. Next, a magnetic field is applied to the target cells, which causes the nanoparticles of iron-oxide core and liposome shallot to move towards them and release their RNA molecules. This noninvasive technique is appropriate for in vitro and even, at least to some extent, in vivo delivery. While physical delivery approaches can provide excellent means to deliver RNA drugs into target cells, it has pros and cons. Henceforth, the selection would largely depend on which system (cell) is being targeted (use-of-the-drug), also depending on type & other characteristics associated with the space-time complexity RNAs are favourable to delivery.

RNA drugs cause all the illnesses.

Overview RNA drugs or RNA-based therapeutics have been buzzwords for the past few years and represent an emerging therapeutic arsenal class. Small but increasingly large RNA molecules are used to bring the efficacy of drugs home and can enhance or suppress expression levels for a gene in your body... helping treat diseases.

Below are examples of RNA drugs for various conditions,

Cancer:

21) Drugs that silence cancer-causing genes, also known as oncogenes, have been developed using RNA interference (RNAi) technology. One such drug is Patisiran, an RNAi therapy that goes after the gene for a protein called transthyretin (TTR), which can build up in people and give rise to an ultra-orphan disease

Known as hereditary TTR-mediated amyloidosis. Patisiran - for the treatment of hATTR amyloidosis.b) A further RNA drug (mRNA vaccine) has been manufactured to prevent cancer. How do these mRNA vaccines work? Once the injected piece of mRNA inside our body that encodes a specific antigen gets into our circulation, this triggers an immune response whereby target cells (cancer cells) get killed. Approval has been granted for using mRNA vaccines for the prevention of cervical cancer, and they are in the development stages to be used as a treatment option against other cancers.

Genetic diseases:

Antisense oligos (ASOs) represent one such class of RNA drugs that can be directed against particular genes involved in genetic diseases. ASOs work by binding to the messenger ribonucleic acid (mRNA) that shuttles genetic directions from DNA, which carries disease-causing mutations in this case, to a cell's protein-producing machinery and prevents production altogether or alter it in such manner as can treat the disease. Spinraza is an ASO drug which already exists and has been approved for treating spinal muscular atrophy, a genetic disease that causes muscle wasting. (b) Single nucleotide genetic diseases have also been treated with mRNA therapy. According to the company, mRNA therapy introduces a synthetic mRNA molecule into the body (in this case, that codes for LCA10 gene GUCY2D) to generate enough functional protein that is missing or defective in patients. An example is the mRNA therapy candidate QR-110 for treating a rare genetic disease called Leber congenital amaurosis 10 (LCA10), which also leads to blindness.

Infectious diseases:

A) mRNA vaccines for infectious diseases like Covid-19, Influenza and rabies. MaintextFigure The mRNA vaccines work by introducing a small piece of messenger RNA (mRNA) in the body to encode for some antigen from the pathogen and elicit a finite role in the human immune system. (b) RNA aptamers are small pieces of RNA which act like drugs and bind to select proteins on the surface of pathogens, thereby blocking their entry into host cells or affecting function. For instance, an RNA aptamer (Nox-A12) is designed to bind a protein called CXCL12, thereby preventing its functions in the growth and spreading of some cancers and maintaining life support for certain viruses such as HIV or SARS-CoV-2.To sum it up, RNA drugs have a bright future in treating many diseases (e.g., cancer, genetic disorders and infectious illnesses, iron/grey]). These drugs that target disease-specific genes or proteins in the body can up-regulate, down-regulate, and modify their expression to treat a specific type of disorder, eventually even cure it.

Conclusions and perspective for the development of RNA drugs.

RNA drugs, also called RNA-based therapeutics, are an emerging class of therapeutic molecules that manipulate gene expression and protein production to treat diseases such as cancer, genetic disorders, and infectious diseases. There are two main classes of RNA drugs being developed to treat diseases: interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs).siRNA drugs degrade targeted messenger RNA (mRNA) molecules and block the production of specific proteins. In contrast, ASOs bind to particular mRNA molecules and obstruct their translation or splicing. Preclinical and clinical results of both types of therapeutic RNA drugs have been encouraging. Fomivirsen, an antisense oligonucleotide (ASO) targeting CMV mRNAs, is the first RNA therapeutic to receive marketing approval from regulatory authorities commencing with CV in 1998. These RNA drugs are subsequently from these medications till Phase III of a clinical trial and approved to enter the dark world: three hereditary transthyretin amyloidosis siRNA-based drugs, primary hyperoxaluria type 1. Although RNA drugs hold great promise, some hurdles still need to be tackled regarding their development and clinical use. Delivery is a big hurdle since RNA drugs are significant, negatively charged molecules that enzymes can break down and quickly flush from the body. However, changes in delivery technologies, including lipid nanoparticles, enabled better endosomal release and increased RNA drug stability. One of the most significant issues is off-target effects - where RNA drugs might interact with a different mRNA molecule and cause changes in protein expression. This would result in off-target toxicity and a narrow therapeutic window of RNA drugs. Common strategies to minimize off-target effects, such as RNA drug design for desired specificity and modified nucleotides, which improve target selectivity, are employed. Such future directions include generating new classes of RNA-based therapeutics, such as circular RNAs and riboswitches, and advancements in delivery technologies that can lower off-target effects by promoting specificity to increase therapeutic efficiency. The emerging field of personalized RNA drugs, designed through sequence-based customization for specific genetic mutations and expression patterns in individual patients, also looks promising to lead to proliferation thereafter (9). Overall, RNA drugs are an up-and-coming field with significant advancements that change entirely how many diseases can be treated. There are still challenges but with research and development.

Underway, more advances will likely be made.

Summary of Review

RNA-based therapeutic medications have become major treatments that use genetic material to control expression patterns linked to various diseases. The review investigates the mechanisms of siRNA and antisense oligonucleotide (ASO) and mRNA therapeutic agents together with their stabilization techniques and delivery breakdowns. New methods of lipid nanoparticle development along with solid-phase synthesis procedures combined with chemical modifications resulted in improved functionality of RNA drugs. The therapeutic agents demonstrate capabilities for managing cancer conditions with genetic disorder and viral infections including vaccines that combat COVID-19. Emerging RNA technology holds great potential for specific medical treatments because researchers continue to overcome delivery barrier issues and avoid unwanted effects from their work. Further research and clinical uses will trigger fundamental changes in modern therapeutic practices during the next decades.

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