Section A-Research paper

Application of Antisense Oligonucleotides: a review

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Abstract

"Antisense oligonucleotides (ASOs)" are a promising class of therapeutic compounds that have attracted a lot of attention lately because of their capacity to target and control gene expression with specificity. This review seeks to provide an overview of the present state of research on the utilization of ASOs in medicine. In-depth discussion is given regarding the mechanism of action of ASOs, which includes their capacity to bind to particular RNA molecules and suppress gene expression. The numerous types of ASOs, such as siRNA, ASOs, and miRNA inhibitors, are also detailed along with their strengths and limitations.

The review highlights the numerous medical uses for ASOs, including as the therapy of cancer, infectious infections, and genetic problems. This field's most recent innovations are addressed, and their potential effects on patient care are assessed. Delivery issues and off-target effects are just two of the difficulties associated with the use of ASOs in medicine that are thoroughly covered.

ASOs represent a promising therapeutic modality with a wide range of applications in medicine. While there are still some problems to be solved, recent breakthroughs in this sector have presented promising potential for the creation of innovative and effective treatments for a number of disorders.

Keywords: antisense oligonucleotides, gene expression, therapeutic molecules, genetic disorders, cancer.

Introduction:

"*Antisense oligonucleotides* (ASOs)" are short, synthetic nucleic acid molecules that bind to specific RNA sequences and modulate gene expression. ASOs were first introduced in the late 1970s as a potential therapeutic approach for genetic disorders, and their application has since expanded to various areas of medicine, including cancer, infectious diseases, and neurodegenerative disorders (1,2).

The basic principle behind ASOs is the inhibition of gene expression by interfering with the mRNA transcription process, either by blocking translation or by degrading the mRNA molecule. ASOs can be designed to target almost any RNA sequence, and their specificity for the target sequence is determined by the complementary base pairing between the ASO and the RNA molecule (3,4).

The mechanism of action of ASOs has been extensively studied over the past few decades. The earliest forms of ASOs were designed to bind to the mRNA molecule and block translation by steric hindrance. However, more recent developments in ASO technology have resulted in the creation of modified ASOs that can also trigger the degradation of the targeted mRNA molecule through a process known as RNase H-mediated cleavage. Other types of ASOs, such as small interfering RNA (siRNA) and microRNA (miRNA) inhibitors, have also been developed that utilize slightly different mechanisms to inhibit gene expression (5).

ASOs have shown promising results in preclinical and clinical studies for various diseases. The first FDA-approved ASO-based drug, fomivirsen, was approved in 1998 for the treatment of cytomegalovirus retinitis in patients with AIDS. Since then, several other ASO-based drugs have been approved, including nusinersen for the treatment of spinal muscular atrophy and inotersen for the treatment of hereditary transthyretin-mediated amyloidosis (4-6).

In recent years, there has been significant interest in developing ASOs as a therapeutic modality for a wide range of diseases. This is due in part to advances in ASO design and delivery technologies, which have allowed for more efficient and targeted delivery of ASOs to specific cells and tissues. Additionally, the ability of ASOs to selectively modulate gene expression makes them an attractive option for diseases that are caused by specific genetic mutations or dysregulated gene expression (5,6).

Despite their potential benefits, there are still several challenges associated with the use of ASOs in medicine. One major challenge is the delivery of ASOs to the target tissue or cell. ASOs are large, charged molecules that are not easily taken up by cells, and therefore, efficient delivery methods must be developed to ensure that ASOs reach their target. Additionally, off-target effects and the potential for immune reactions to the ASO molecule must also be carefully considered (4-6).

This review will provide an overview of the current state of research on the application of ASOs in medicine. The mechanism of action of ASOs, the different types of ASOs, and their strengths and limitations is discussed as well. Finally, the challenges associated with the use

of ASOs in medicine and provide recommendations for future research in this field are presented.

Mechanism of ASOs:

ASOs are artificial compounds that have the ability to attach to RNA sequences and control gene expression (7). The capacity of ASOs to detect particular RNA sequences and create stable Watson-Crick base pairs with them underlies their mode of action, which results in the suppression or regulation of RNA function (8).

ASOs can operate using a variety of mechanisms, such as:

1. Degradation of RNA: ASOs can attach to specific RNA sequences and call for the cellular endonuclease RNase H, which cleaves the RNA molecule at the location of the ASO-RNA hybrid. As a result, the targeted RNA molecule degrades, which causes the expression of genes to be downregulated (9).

2. Modulation of alternative splicing events: ASOs can be made to specifically target pre-mRNA sequences. ASOs can change the splicing pattern of the mRNA and produce alternative protein isoforms by binding to particular exon or intron regions (10).

3. Inhibition of translation: ASOs can also stop translation by attaching to mRNA molecules, obstructing the ribosome binding site, or causing structural alterations in RNA that obstruct effective translation (11).

ASOs have demonstrated potential as therapeutic agents for a range of illnesses, including cancer, viral infections, and genetic abnormalities (12). Spinal muscular atrophy (SMA), a rare genetic condition characterized by the loss of motor neurons, is one instance of how they are used in treatment. It has been demonstrated that ASOs created to boost SMN protein synthesis are successful in restoring motor function in SMA patients (13).

Viral diseases including HIV and the hepatitis B virus (HBV) have also been treated with ASOs. ASOs created to bind to the viral RNA genome and prevent viral replication in the case of HIV have showed promise in preclinical experiments (14). Similar results have been shown with ASOs that target the HBV RNA in both human patients and animal models (15).

Despite having therapeutic potential, ASOs' poor pharmacokinetic characteristics and off-target effects have made them difficult to deploy in clinical settings. Chemical alterations and combination with delivery agents such lipid nanoparticles and peptides have been used to enhance ASO delivery and lessen non-specific side effects (16). ASOs may now be designed to target specific gene sequences with great accuracy thanks to developments in genome editing technologies like CRISPR-Cas9 (17).

In conclusion, the function of ASOs is to bind to particular RNA sequences and control the expression of genes by a variety of processes, such as RNA degradation, splicing regulation, and translation inhibition. ASOs have a wide range of therapeutic applications that could be used, and continuing research is being done to increase their clinical efficacy and safety.

Types of ASOs:

ASOs can be created to target DNA, mRNA, and microRNA (miRNA) as well as other elements of the gene expression pathway. The structure, duration, and mechanism of action of the various ASO types employed in these applications can differ.

1. DNA ASOs, first The promoter or enhancer regions of a target gene are where DNA ASOs are intended to hybridize to the genomic DNA, inhibiting transcription and lowering expression. These ASOs typically span 18 to 25 nucleotides, making them longer than RNA-targeting ASOs (18). Oncogenes, such as the MYC oncogene in multiple myeloma, have been targeted by DNA ASOs in cancer therapy (19).

2. mRNA ASOs: mRNA ASOs are made to bind to a target mRNA molecule's coding area, preventing translation and lowering protein production. These ASOs typically have a length of 8 to 30 nucleotides (20), which is shorter than DNA ASOs. Numerous disorders, such as Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA), have been treated with mRNA ASOs (21,22).

3. Splice-Switching Oligonucleotides antisense Splice-switching ASOs are made to bind to pre-mRNA and modulate splicing, changing the ratio of alternatively spliced isoforms and changing how proteins are expressed as a result (23). For example, in DMD and SMA, these ASOs can be utilized to fix mutations that interfere with regular splicing (24,25).

4. MicroRNA ASOs /miRNA ASOs: miRNA ASOs are created to bind to endogenous miRNAs, disrupt their activity, and increase the expression of their target genes as a result. These ASOs can be utilized to treat conditions like cancer and cardiovascular disease, where the pathogenesis of the condition is aided by the downregulation of a particular miRNA (26,27).

5. Aptamers Similar to antibodies, aptamers are single-stranded nucleic acid molecules that have a high affinity and specificity for binding to particular targets (28). Proteins, tiny molecules, cells, and other compounds can all be targeted by aptamers. ASOs and other therapeutic compounds can be delivered by aptamers or employed independently as therapeutic agents (29).

In conclusion, different types of ASOs have different uses in the control of gene expression, and each type's mechanism of action and therapeutic potential are different. A variety of methods have been used in the design of ASOs for therapeutic purposes, including DNA ASOs, mRNA ASOs, splice-switching ASOs, miRNA ASOs, and aptamers. The efficacy, specificity, and safety of ASOs for clinical usage in a wide variety of disorders are the subject of ongoing research.

Applications of ASOs:

A number of disorders have showed tremendous promise for ASOs as a treatment strategy. They are a desirable choice for the treatment of cancer and other acquired diseases, as well as

hereditary abnormalities, due to their capacity to target specific genes and modify their expression. We go over a few of the uses for ASOs here.

Genetic Conditions

Treatment of genetic illnesses is one of the most promising uses for ASOs. ASOs can reduce or eliminate disease symptoms by correcting or moderating the expression of a gene by targeting the gene. ASOs, for instance, have been utilized to treat spinal muscular atrophy (SMA), a hereditary condition that weakens and atrophyes the muscles. The antisense oligonucleotide nusinersen was demonstrated in a clinical trial to enhance motor function and increase survival in newborns with SMA (30). Another genetic condition that affects the muscles, Duchenne muscular dystrophy (DMD), has also been treated with ASOs. Antisense oligonucleotide systemic treatment caused exon skipping and restored dystrophin expression in dogs with DMD in a preclinical study (21).

Cancer

It has also been investigated whether ASOs could be used to treat cancer. ASOs have the ability to suppress tumor growth and cause cell death by specifically targeting genes that are overexpressed or mutated in cancer cells. In preclinical models, it has been demonstrated that an antisense oligonucleotide that targets the oncogene BCL-2 can cause cancer cells to undergo apoptosis and stop the formation of tumors (31). Targeting microRNAs, small non-coding RNAs that control gene expression and have been linked to cancer, has also been done using ASOs. In a preliminary investigation, mouse lung cancers were suppressed by systemically delivering tumor suppressor microRNA mimics in a neutral lipid emulsion (27).

Virus and Infections

It has also been investigated whether ASOs could be used to treat infectious disorders. ASOs can prevent viral replication and lower viral load by specifically targeting viral RNA. For instance, it has been demonstrated that an antisense oligonucleotide that targets the RNA of the hepatitis C virus (HCV) prevents HCV replication both in vitro and in vivo (32). In order to stop bacterial growth, ASOs have also been applied to target bacterial RNA. An antisense oligonucleotide that targets the RNA of the bacterial pathogen Staphylococcus aureus caused a considerable decrease in bacterial load in infected mice in a preclinical research (33).

Delivery of ASOs

Effective transport to the target cells and tissues is one of the difficulties in the development of antisense oligonucleotide therapies. Large molecules known as ASOs are unable to easily pass across cell membranes and are rapidly degraded by nucleases in tissues and the blood.

The utilization of delivery systems such liposomes, nanoparticles, and conjugates, as well as chemical modification of the oligonucleotide backbone to increase stability and pharmacokinetics, have all been proposed as solutions to these problems.

Chemical Modification

ASOs' stability, pharmacokinetics, and binding affinity to target RNA can all be improved by chemically altering the oligonucleotide backbone. Phosphorothioate linkages, 2'-O-methylation, locked nucleic acid (LNA) substitutions, among other things, are some of these alterations (34).

Delivery Vehicles

ASOs can be more effectively delivered to cells and distributed throughout the body by using delivery systems such liposomes, nanoparticles, and conjugates. ASOs can be encapsulated and more easily absorbed by cells via lipid-based particles called liposomes. ASOs can also be enclosed in nanoparticles, which can be designed to target particular cells or tissues. ASOs can also be delivered to particular cells or tissues using conjugates, such as peptide or antibody conjugates (35).

Therapeutic Trials

Many antisense oligonucleotide therapies are now undergoing clinical studies, and a number of them have been approved for use in patients. Fomivirsen, a phosphorothioate oligonucleotide that targets the RNA of the cytomegalovirus and was approved by the FDA in 1998 for the treatment of cytomegalovirus retinitis in individuals with AIDS, was the first antisense oligonucleotide therapy to be approved (36).

Other antisense oligonucleotide therapeutics that have received approval include eteplirsen, an oligonucleotide that induces exon skipping in the dystrophin gene, and mipomersen, an oligonucleotide that targets apolipoprotein B and is used to treat familial hypercholesterolemia (37, 38).

A number of disorders have showed tremendous promise for ASOs as a treatment strategy. The creation of effective delivery methods is still difficult, though. The cellular absorption and biodistribution of ASOs have been improved by chemical alterations and delivery systems such liposomes, nanoparticles, and conjugates. Many antisense oligonucleotide therapies are now undergoing clinical studies, and a number of them have been approved for use in patients. ASOs are likely to become a key tool in the treatment of diseases as this field of study develops.

Challenges and Future Directions:

ASOs provide a wide range of benefits and possible uses, but there are still a number of issues that need to be resolved. The transport of these compounds to their target areas in vivo is a significant difficulty. As was previously indicated, oligonucleotides can be quickly eliminated from circulation and are prone to nuclease-mediated degradation. Therefore, to prevent these molecules from degrading and to improve their uptake by target cells, effective delivery mechanisms are required.

The delivery problem has been addressed using a variety of strategies, such as conjugation with nanoparticles, liposomes, and other carriers. Guo and Huang, for instance, showed that nanoparticle encapsulation of ASOs could improve their stability and extend their circulation time in vivo. Additionally, these nanoparticles' surface changes might help with their targeted transport to particular tissues or cells.

Off-target effects, which may cause unintentional gene silencing and negative outcomes, are another problem. Off-target effects can still happen even though ASOs specificity can be improved by changing their chemical structure or choosing the right target sequences. To reduce off-target effects and ensure the safety and efficacy of these compounds, thorough design and validation are essential.

Other difficulties include the short-lived nature of their actions and the possibility for immune system activation, in addition to delivery and off-target consequences. The therapeutic effects of ASOs may be limited by the fact that they are often eliminated from circulation within hours or days. Additionally, the immune system has the ability to identify these molecules as alien and launch immunological reactions, which might diminish their effectiveness or have negative effects.

ASOs have demonstrated excellent results in preclinical and clinical trials despite these difficulties, showing their promise for a variety of therapeutic applications. The therapeutic applicability of this technique is highlighted by the recent approval of numerous antisense oligonucleotide-based medications, including nusinersen and eteplirsen, for the treatment of Duchenne muscular dystrophy and spinal muscular atrophy, respectively.

Future advancements in antisense oligonucleotide-based therapeutics may include the creation of more effective delivery mechanisms and the discovery of novel target sequences. ASOs may also improve the therapeutic effects of other treatment methods, such as gene editing or immunotherapy, and facilitate the creation of customized medicine.

In conclusion, ASOs are a promising class of medicines that have the potential to be used to treat a variety of illnesses. Their therapeutic value is highlighted by the recent approval of numerous antisense oligonucleotide-based medications, despite the difficulties related to their transport, specificity, and immune reactions. Personalized medicine could be made possible by the creation of more effective delivery methods and their interaction with other therapeutic techniques.

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