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Review article

Recent advancements, challenges and strategies of antisense oligonucleotide in drug delivery system: An overview

P Magesh, M Devi Vasantha, R Titus Bruno, M Dharani Dharan, S Nagalakshmi*

Department of Pharmaceutics, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India

Corresponding author: S Nagalakshmi, ✉ nagalakshmi.s@sriramachandra.edu.in, **Orcid Id**: https://orcid.org/0009-0005-5787-7377

Department of Pharmaceutics, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India

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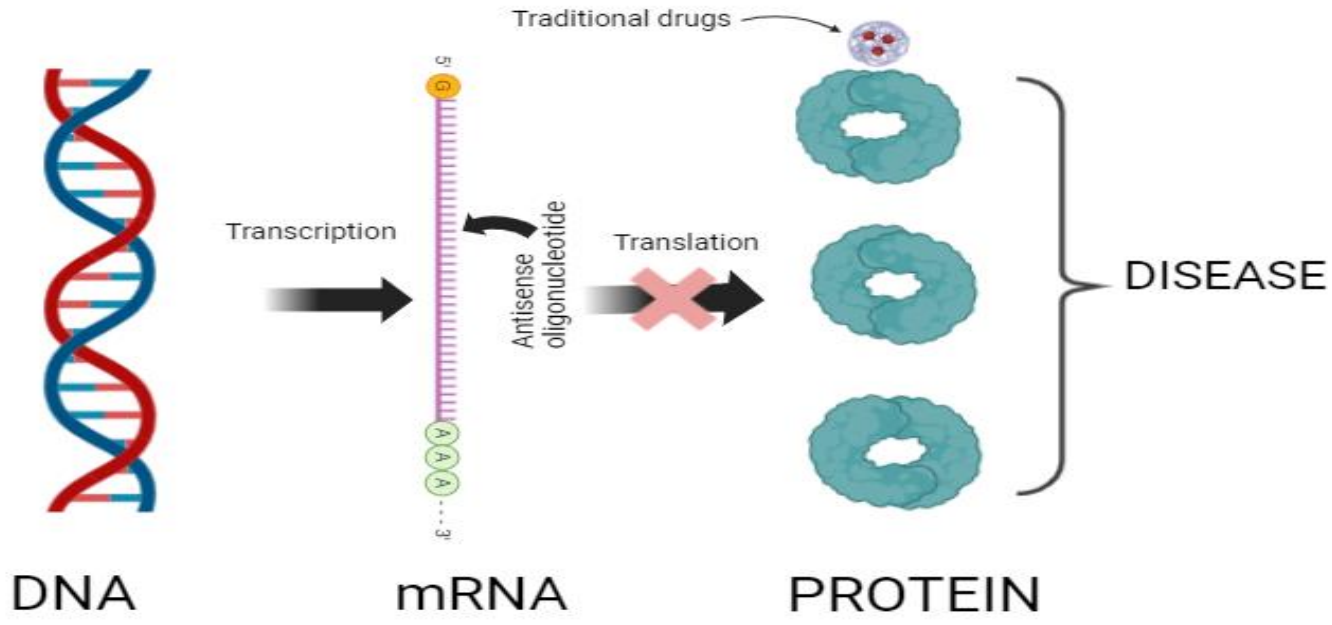
ABSTRACT

Antisense oligonucleotides (ASOs) are small synthetic nucleic acids designed to bind RNA and modulate gene expression. They have significant therapeutic potential for treating genetic and acquired diseases, especially in cancer and hereditary disorders. Their primary mechanisms include RNA cleavage, mediated by RNase H1 or RNA interference (RNAi), and RNA blockage via steric hindrance or splice modulation. First-generation modifications, such as phosphorothioate linkage, and more advanced forms like locked nucleic acids (LNA) and peptide nucleic acids (PNA) have improved their pharmacokinetics and binding affinity. ASOs have achieved success in treating diseases like spinal muscular atrophy and Duchenne muscular dystrophy, with several FDA-approved drugs.



Keywords: Antisense oligonucleotides (ASOs), Mechanism of action, Lipid nanoparticles (LNP), Pharmacokinetics, FDA Approved formulations.

INTRODUCTION

The acquisition of therapeutic targets and ailments biomarkers has been made possible by the association between diseases and variations in gene expression, which transformed clinical and molecular research. Since then, oligonucleotide therapies have been created to precisely quiet, repair or change the expression of genes associated with genetic diseases and cancer [1]. Among the therapeutics are siRNA, microRNA, and antisense oligonucleotides that hinder RNA, both coding and noncoding; formerly "undruggable" proteins that are not treated by traditional small molecules are controlled using RNA interference, which controls protein production [2].

Oligonucleotides (ONs) are single or double-stranded segments of modified nucleic acids which is employed as potential therapeutic agents. The special quality of ONs is their ability to bind target through Watson-Crick base pairing by selectively targeting RNA [3]. ONs are a broad category of nucleic acid-based treatments that include aptamers, anti-miRNA, siRNA, miRNA mimics, ASOs. As of right now, the US FDA has approved eleven ON-based medications to treat genetic diseases like familial amyloid polyneuropathy, spinal muscular atrophy, and Duchenne muscular dystrophy [4].

Mechanism of Action

Most antisense medications studied in clinical settings work in a way that is dependent on RNase H. The RNA strand of an RNA/DNA duplex is hydrolyzed by RNase H. A significant amount of protein and mRNA expression can be down-regulated by 80–95%, when targeted RNA expression is reduced using oligonucleotides in conjunction with RNase H. Furthermore, when directed to almost any region of the mRNA, RNase H-dependent oligonucleotides can suppress protein expression, compared to steric-blocker oligonucleotides [5].

Table 1: mechanism of action

Mechanism	Subclass	Explanation
RNA cleavage	RNase H1 mediated cleavage	RNase H1 binds to the ASO-mRNA heteroduplex and cleaves the target mRNA.
	RNA interference (RNAi)	SiRNA-mediated mRNA degradation occurs through RISC.
RNA blockage	Steric hindrance	ASO-mRNA heteroduplex inhibits the interaction of mRNA and ribosomes, therefore prevents protein interpretation.

RNA Cleavage

RNase H1 mediated degradation

Oligonucleotides bind to RNA to produce ASO-RNA complex, which act as substrates to the cytoplasmic RNase enzymes**.** The RNA heteroduplex is broken down by RNases. RNase H1 activity is aided by the unaltered nucleotide sequence in the center of the gapmer design, while the changed nucleotides surrounding it improve the enzymatic resistance and binding affinity of the gapmer. The majority of FDA-approved medications use RNases to carry out their antisense function [6].

RNA interference (RNAi) mechanism

Exogenous siRNAs are 22-nucleotide long, double-stranded RNA molecules with 2-nucleotide present at 3′ position of each strand. [7]. these siRNA integrated into RISC, whereby one strand is broken down by the Argonaute 2 (Ago 2) enzyme. Whereas another strand guides RISC to the complementary mRNA, allowing Argonaute 2 enzyme to cleave mRNA, thereby inhibiting expression of genes [8].

Steric Blockage

Mechanism of translation arrest due to steric hindrance

ASO attach to the specific RNA, obstructing the interaction or assembly of the 40S ribosomal subunit with the 60S subunit, leading to a blockage of translation. There is a direct relationship between steric hindrance and ASO binding affinity. Increased binding affinity leads to stronger combination of ASOs and RNA, leading to the blockage of translation. Additionally, chemical ASOs, typically 20–25 nucleotides in size, are intended to inhibit gene expression by binding miRNA and preventing its association with mRNA through a steric hindrance mechanism [9].

Splice modulation based mechanism

Splice switching- based mechanism are classified into: (1) exon skipping and (2) exon inclusions. When exon skipping occurs, ASO attach themselves with preliminary mRNA and repair malfunctioning reading frame, and generate effective proteins. Whereas in exon inclusion, Antisense oligonucleotides attach with preliminary mRNA location and block both the splicing factors and spliceosome [10].

Modification of ASO

Prior research has employed unmodified ASOs to target RNA. Nevertheless, unaltered ASOs are susceptible to nuclease degradation and unaltered ASOs big size and charge limit their passive diffusion [11]. Therefore, chemically modified ASOs in an effort to enhance their effectiveness and enzymatic stability while minimizing immune response and associated damage.

The initial backbone chemistry capable of effectively modifying the phosphodiester linkage is phosphorothioate [12]. The compound shows a wide spectrum of activity when oxygen atom is substituted with a sulfur atom. This molecule forms a stable duplex with mRNA and resistance to nucleases. The primary drawbacks of phosphorothioates are their low plasma drug concentrations, diminished affinity for target mRNA, and potential side effects, including coagulation disorders and immune system activation. Second-generation molecules are made up of nucleotides that have structural similarities to both RNA and DNA. At smaller doses, these medications have therapeutic actions, by protecting the medication from harmful nucleases [13].

Third-generation ASOs were synthesized by chemically altering antisense oligonucleotides' furanose ring, as well as nucleotide and phosphate linkage alterations. Peptide nucleic acid (PNA), locked nucleic acid (LNA), and morpholino phosphoramidates (MF) are the three third-generation antisense oligonucleotides that are most commonly utilized [14].

Delivery System

Delivery using enhanced stabilization chemistry

To deliver siRNA, Alnylam made use of enhanced stabilization chemistry (ESC). siRNAs bind to N-acetyl galactosamine (GalNAc) in the ESC. GalNAc also lessens immunological stimulation and enhances the stability of siRNA conjugates in lymphatic system, plasma, and hepatocytes [15].

Delivery using polymer-based nanoparticles

Delivering ASOs has been done using a variety of polymeric nanoparticle-based delivery methods, such as Poly (lactic-co-glycolic) acid, and Polyethylenimine. Through their "proton sponge" effect, PBAE and PEI improve ASO delivery while reducing endosomal entrapment [17]. Unfortunately, toxicity from high cationic charge and numerous non-specific interactions between tissue and serum proteins hinder progress of these delivery systems towards clinical usage. Conversely, PLGA has been frequently employed to create nanoparticles and distribute ASOs since it is a biocompatible and FDA-approved polymer [18].

Lipid-Based delivery systems

ASOs and siRNAs have been widely delivered via lipid-based delivery methods, including lipid nanoparticles (LNP), liposomes and lipoplexes. Polyethylene glycol (PEG) coating is commonly used to coat LNPs, leading to an extended duration of blood circulation [19]. LNPs also exhibit enhanced permeability and retention (EPR) impact accumulation within the carcinoma microenvironment. Based on LNP, Patisiran's siRNA formulation was developed [20].

ADME Characteristics of ASO

Absorption and Distribution

The rate of absorption of ASOs are influenced by several physicochemical properties. Distribution is influenced by administration method, tissue blood perfusion, free-drug concentration, tissue binding, local pH, and cell membrane permeability [21]. In order to maximize bioavailability, the majority of ASOs are given intravenously (IV). As a result, the drug rapidly diffuses into highly vascularized organs such as the liver, kidneys, and spleen, whereas delivery of ASOs into tissues such as heart, muscles and lungs require periodical dosing. It has been demonstrated that delivery of oligonucleotides by subcutaneous (SC) injections are a feasible alternative for IV infusions.

Direct ASO delivery has been effective in reaching locations that are inaccessible via IV or SC administration. Fomivirsen can be administered locally into the eye through intravitreal injection, which allows for direct drug distribution to the retina with a relatively tiny dosage [22]. Administration of ASOs by Intrathecal route has been promising method than intravenous (IV) and subcutaneous (SC) routes in delivering ASOs to less perfusion tissues as it minimizes frequent dosing, although it is more invasive [23].

ASO distribution through intranasal administration has also been investigated for CNS diseases. According to certain theories, ASO molecules can migrate via the rostral migratory stream, the trigeminal and olfactory nerve pathways, and intranasal delivery. This method delivered siRNAs to the tissues like brain through the intranasal administration [24].

As a result of low gastrointestinal absorption, oral delivery of ASOs has proven difficult. ASO bioavailability can differ based on physicochemical properties. After absorption, ASOs reversibly bind to lipids and proteins in plasma and tissues, enabling their circulation. Then ASOs by the mechanism of diffusion or endocytosis reach the cytoplasm and finally exclude from vesicles, and may require further translocation to the nucleus, based on their location of target, ASOs require effective concentration to provide the required therapeutic response. After entering cells, ASOs maintain prolonged half-life (1–4 weeks) and extended cellular function [25].

Metabolism of ASO

ASOs are metabolized by nucleases, which are broadly present in cells across the majority of tissues. However, the rate of metabolism of ASOs differs in species and is mostly dependent on the chemical backbone. Endonucleases cleave the inner portion of the ASO by recognizing the 2′-hydroxy group on the ribose, while exonucleases target the terminal ends of the ASO, effectively cleaving at the 3′-hydroxy group [26].

Excretion

ASOs are mainly eliminated in urine or feces either in the form of intact therapeutics or as cleaved short fragments, depending on their charge, polarity, and hydrophilicity. The rate at which proteins bind has an impact on elimination. ASOs are spared from glomerular filtration and their primary route of clearance—renal clearance—is slowed down when they bind to circulating proteins. There has been a suggestion that PMO's short plasma half-life and rapid renal clearance are caused by its poor protein binding [27].

Clinical Application

Antisense oligonucleotide in anti-arthritic agent

Researchers Akhavein and colleagues investigated the potential of microencapsulated ASOs as a powerful anti-inflammatory agent, specifically designed to target and reduce nuclear factor-kappa B (NF-kB). The study found that treatment with microencapsulated antisense oligonucleotides led to a notable reduction in NF-kB activity [28].

Makalish and colleagues investigated the effects of the antisense oligonucleotide Cytos-11 on the inhibition of TNF-α gene

expression in a rat model. Research has shown that Cytos-11 ASOs effectively reduce TNF-α expression, which consequently lowers peripheral blood concentrations, alleviates joint inflammation, and slows the progression of pannus formation. The outcomes contrasted favorably to adalimumab [29].

The effect of ASOs in inhibiting the proliferation of rheumatoid synovial fibroblasts was described by Morita et al. Interleukin-1β, the cause of rheumatoid arthritis (RA), was released by fibroblast cells. ASOs have been used to target messenger RNA in order to treat interleukin-1β. Cells treated with oligonucleotide showed both mRNA and protein levels of proliferating cell nuclear antigen, suggesting that the antiproliferative effect was attained through a distinct therapeutic strategy [30].

Anti-inflammatory agent

Donner et al. reported on a novel antisense oligonucleotide-based therapy for doxorubicin-induced nephropathy and renal inflammation. According to experimental and clinical research, nephropathy, renal damage, and inflammation are associated with CD40 activation. One unique approach to treating unilateral ureter blockage and damage in animal models is the suppression of renal CD40 expression. In this instance, 2.5 CD40 ASO was given to the experimental animals, which inhibited CD40 mRNA levels in the kidney between 75 and 90%. Thus, our data clearly suggests CD40 ASO as a useful treatment for doxorubicin-induced renal inflammation and nephropathy [31].

Zorzi et al. explored a novel strategy for treating inflammatory bowel disease using antisense oligonucleotides (ASOs) that target Smad7. In the case of inflammatory bowel disease (IBD), Smad7 ASOs have been shown to inhibit TGF-β1. These results indicate that Smad7 ASOs are both safe and well-tolerated in patients with Crohn's disease [32].

Anticancer agents

In a mouse model, Vanderborght et al. investigated the impact of antisense oligonucleotides targeting carcinogenesis and fibrosis. Hypoxia-inducible factor (HIF) is important in development of cancer. The authors explored the potential therapeutic effects of ASOs in tumor microenvironment's fibrotic, inflammatory, and neoplastic components. Their results indicate that ASO isoforms are not viable therapeutic options in the treatment of hepatocellular carcinoma [33].

BCL-2 has been shown to have potential as a tumor treatment by Ciardiello et al, ASOs regulate the endogenous expression of genes by binding to certain mRNAs. As a result, the growth and spread of the tumor may slow the tumor cell [34].

Table 2: FDA approved formulation

Category	Drug Name	Developer	Therapeutic Indication	FDA/EMA Approval Year
ASO	Fomivirsen	Ionis Pharmaceuticals	CMV retinitis in AIDS patients	1998
	Mipomersen	Ionis Pharmaceuticals	Familial hypercholesterolemia	2013
	Eteplirsen	Sarepta Therapeutics	Duchenne muscular dystrophy	2016
	Nusinersen	Ionis Pharmaceuticals	Spinal muscular atrophy	2016
	Defibrotide	Jazz Pharmaceuticals	Veno-occlusive disease in the liver	2016
	Inotersen	Akcea Therapeutics and Ionis Pharmaceuticals	Nerve damage in adults with hereditary transthyretin-mediated amyloidosis	2018
	Volanesorsen	Ionis Pharmaceuticals/ Akcea	Familial chylomicronemia syndrome	2019
	Golodirsen	Sarepta Therapeutics	Duchenne muscular dystrophy	2019
	Viltolarsen	NS Pharma	Duchenne muscular dystrophy	2020
	Casimersen	Sarepta Therapeutics	Duchenne muscular dystrophy	2021
siRNA	Patisiran	Alnylam Pharmaceuticals	Hereditary transthyretin-mediated amyloidosis	2018
	Givosiran	Alnylam Pharmaceuticals	Acute hepatic porphyrias	2019
	Inclisiran	Alnylam Pharmaceuticals	Hypercholesterolemia	2021
	Lumasiran	Alnylam Pharmaceuticals	Primary hyperoxaluria type 1	2020
	Vutrisiran	Alnylam Pharmaceuticals	Hereditary transthyretin-mediated amyloidosis	2022
Aptamer	Pegaptanib	OSI Pharmaceuticals	Hereditary transthyretin-mediated amyloidosis	2004

CONCLUSION

Antisense oligonucleotides (ASOs) regulate gene expression by targeting RNA, providing therapies for genetic, inflammatory, and cancerous diseases. FDA-approved treatments for conditions like Duchenne muscular dystrophy and spinal muscular atrophy showcase their effectiveness. Advances such as locked nucleic acids (LNAs) enhance stability and efficacy, though delivery challenges and immune responses remain. Promising delivery methods include nanoparticle and lipid-based systems to improve bioavailability and targeting. ASOs' adaptability to both coding and noncoding RNAs underpins their potential in personalized medicine. Future studies of antisense oligonucleotides (ASOs) will focus on improving delivery systems, such as nanoparticle and lipid-based carriers, to enhance tissue targeting and reduce immune responses. Research will also aim to refine chemical modifications for better stability, efficacy, and safety, enabling broader applications in personalized medicine.

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