

# Antisense Oligonucleotides: Pharmacology and Delivery Strategies

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## ABSTRACT

Antisense oligonucleotide therapy is a dominant drug discovery approach that can explicitly modify protein synthesis through numerous mechanisms. The limitations of antisense oligonucleotide (ASO) therapy in delivery strategies have been overcome in recent years with different ligands carriers, as well as, through nanocarriers. ASO therapy was successfully applied towards targeting a wide range of therapeutic areas. There is an expanding enthusiasm in extending the utilization of antisense compounds to numerous different diseases due to their safe and potential therapeutic outcomes. Thus, the present review attempted to elaborate on the fundamental idea of antisense technology, approaches, and safe and effective delivery methods.

**Keywords:** Antisense, Delivery, Mechanism, Oligonucleotide, Pharmacokinetics, Pharmacology.

## 1. INTRODUCTION

According to the recent designation, a synthetic single-stranded nucleic acid chain with 8 to 50 nucleotides is termed as an ASO.<sup>1</sup> In 1978, Stephenson and Zamecnik first discovered the process of gene inhibition with ASO to stop viral replication in cell culture. ASO binds through standard base pairing to their complement RNA, altering RNA function and thereby hamper the gene expression. They can be utilized to modify the synthesis of proteins and alter the synthesis of toxic proteins.<sup>2</sup> The present review discussed the mechanism of ASO action, approaches, pharmacology, and various effective delivery methods.

## 2. ANTISENSE APPROACH

### 2.1. Antisense Deoxyribonucleic Acid (ADA)

Primary approach for ASO is utilizing single-stranded antisense deoxyribonucleic acids with 15 to 25 base pairs. They are intended to be correlative to discrete destinations on a particular targeted mRNA forming DNA:RNA hybrids. The mechanisms to restrain transcription process consist of directly binding to DNA, through formation of triple-stranded helix and combining with transcription factors.<sup>3</sup>

### 2.2. Antisense Ribonucleic Acid (ARA)

Another approach uses ribonucleic acids, which ties to a particular target mRNA, which obstructs the formation of a particular protein. This methodology requires acquaintance and production of a small part of the complete gene sequence.<sup>4</sup>

There are two general classes of ASOs utilized in antisense therapy. First is the single-stranded ASO that activates enzyme RNase H, which causes degradation of the target RNA and another class is the double-stranded synthetic oligonucleotides that form RNA-induced silencing complex which then degrade the target RNA.<sup>5</sup>

### 2.3. Advantages of ASOs

- ASOs are a lot simpler to concentrate on than numerous different approaches to treat different diseases.
- Extent of sensitivity can be measured with ease and can be modified as needed.
- ASOs can be controlled and conveyed as needed without causing harm to healthy cells.
- They can be used in targeted drug therapies in place of conventional drugs with huge biodistribution and toxicities.

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- ASOs are potential adjuvant in the treatments of neurological disorders, AIDS, cancer, diabetes, hepatitis, etc.

## 2.4. Disadvantages of ASOs

- Complex process and high cost of production.
- Intricacy in delivery into the body, especially through a pill as the human body has a characteristic barrier against it.
- Rapid excretion, deprivation by enzyme nucleases, and degradation by phagocytes in liver and spleen.
- Slow diffusion and binding in an extracellular fluid environment.
- Inevitable toxic effects because ASOs regulate both mutant and normal alleles.
- Challenge to determine the correct composition and dosage regimen to reduce unintended side effects caused by an alteration in genes.

## 3. MECHANISM OF ASOS

ASOs bring about the development of hetero-duplex ASO-mRNA complex, when combined and incorporated with the complement target mRNA that leads to activation of RNase H enzyme or steric hindrance of ribosomal subunit binding. This mechanism causes debasement of ASO-mRNA complex and ultimately target protein knockdown. Oligonucleotide with RNase H enzyme activation, when targeted to any locale on the mRNA, causes its damage and debasement.

Further mechanisms of working of ASOs is by inward bounding to DNA/RNA directly into the nucleus and by altering the progress of mRNA, inhibition of 5'-cap formation, translation process, and activation of RNase enzyme.<sup>6</sup>

## 4. SYNTHESIS AND DESIGN

The initial phase in designing ASOs is recognizing the useful sequences for target inhibition, which should be a complement to that specific gene, and available for forming bound ASO complex. An ASO must be coordinated and designed in a structure that conveys adequately and contradicts deprivation *in vivo*. The affinity of ASOs to the target mRNA is a measure of the stability of the nucleic acid hybrid. The enhanced affinity of ASOs has a high probability of gene repressing action.<sup>7</sup>

## 5. CHEMICAL MODIFICATIONS

Synthetically designed unmodified ASOs have hostile and unfavorable characteristics that cause difficulties in

their development. Degradation by nucleases, poor entry into cells, and low binding affinity for integral sequences are major hindrances. To overcome these obstructions, the backbones of oligonucleotides were chemically modified and arranged by the addition of phosphodiester bonds, ribose sugar bases, and nucleobases. ASOs are progressing due to increased chemical modification, which enhances their *in vivo* half-life, distribution throughout the body, potency, and decreases toxicity.<sup>8,9</sup>

### 5.1. First Generation ASOs

In these ASOs, sulfur is substituted in place of non-bridging phosphoryl oxygen of DNA. This substitution protects the ASOs from nuclease digestion and increases half-life with acceptable tissue distribution. First-generation ASOs are potent and well-tolerated, typically with low toxicity.

### 5.2. Second Generation ASOs

These ASOs involves chemical modification of the ribose phosphodiester backbone linkage, or the heterocycle, or the sugar group. Substitution of 2'-position with an electronegative group, such as, 2'-O-methyl, 2'-O-methoxyethylgroup, or 2'-O,4'-C-methylene bridge gives an RNA, like C3'-endo conformation to the modified oligonucleotide that increases affinity and stability to a greater extent. These ASOs exhibit much improved pharmacokinetic characteristics.

### 5.3. Third Generation ASOs

Addition of peptide nucleic acids to chemically modify the ASOs guarantees further enhancements later on. An example is the phosphorodiamidate morpholino oligomers, which is designed with a morpholine ring in place of ribose ring that has phosphoroamidate linkages.<sup>10-13</sup>

## 6. PHARMACOKINETICS

### 6.1. Route of Administration

The primary route for systemic delivery of ASOs is by parenteral injection via subcutaneous (SC) injection or intravenous (IV) infusion.

### 6.2. Absorption and Distribution

On administration by subcutaneous injection, ASOs are quickly absorbed with highest plasma concentrations within 3 to 4 hours and rapidly declines by distribution phase, in which ASOs relocate from circulation to tissues

in minutes or a few hours. The elimination phase then is much slower. Many biological hindrances remain between the oligonucleotide and its site of action. Modified ASOs broadly bound to the plasma proteins (85%), facilitates uptake in tissues, increase tissue half-life, prevent loss of drug in renal excretion, and modulate protein binding. A deficit charged oligonucleotides bound inadequately to target mRNA, which further leads to its rapid excretion.<sup>14,15</sup> Most ASOs are unable to cross the vital barriers, such as, blood-brain barrier and placenta. Nevertheless, the delivery in the cerebral spinal fluid may result in extensive distribution into spinal cord and brain tissue.<sup>16</sup>

### 6.3. Metabolism and Clearance

Compared with bolus administration, gradually IV infused ASOs brought about considerably more uptake in the liver, incriminating a saturated uptake process. Liver, kidney, lungs, bone marrow, spleen, lymph nodes, and adipocytes were reported to have the highest accumulated concentrations.<sup>4</sup> The attached ASO influence the metabolism of the target RNA.<sup>17</sup>

## 7. DELIVERY OF ASOS

ASO do not readily permeate into the cell membranes due to the lipophilic lipid bilayer. Consequently, suitable chemically-based methodologies for delivery of ASO possibly will be supportive in ASO therapeutics. Endocytosis is a common process for cellular uptake of nucleotides. It depends on the cells the regular process of receptor interceded endocytosis. The downside of this strategy is the long time period and inefficient outcomes.<sup>18,19</sup> The following are several strategies developed for the effective delivery of ASOs.

### 7.1. Micro-Infection

In this method, the ASO molecule is injected into the cell. This method is very reliable, but only about 100 cells can be infused in one day, since it requires the highest accuracy to inject a smaller molecule in very small cell.<sup>20</sup>

### 7.2. Liposome Encapsulation

This method is the best strategy for huge amounts of ASOs in lipid vesicles, yet additionally expensive one. Liposome encapsulation can be accomplished by utilizing those substances which can cross phospholipids bilayer membrane of the cell. Subsequent liposome converges with the cell layer permitting the entrance of antisense into the cell.<sup>21,22</sup>

### 7.3. Electroporation

This is the conventional method of transferring an antisense nucleotide sequence into a cell. The antisense molecule is permitted to go through the cell film under the influence of a shock that is applied to the cells.<sup>23,24</sup>

### 7.4. Antisense Polygalacturonase Gene

The enzyme polygalacturonase is concerned with the breakdown of pectin, a component of cell walls. To slow the softening process, the Flavr Savr utilizes antisense technology to block polygalacturonase enzyme production. The antisense polygalacturonase-RNA is complementary to the mRNA produced by normal polygalacturonase genes, which will restrain the polygalacturonase enzyme by disabling their mRNA. This partial double-stranded complex is also rapidly degraded.<sup>25</sup>

### 7.5. Polycationic Cell-Penetrating Peptides (PCPP)

These PCPP primarily bind to glycosaminoglycans present on cell surface, followed by endocytotic uptake, and which ultimately releases the loaded ASO to the targeted cytosol. Generally, PCPPs deliver oligonucleotides with uncharged peptide nucleic acids, morpholino ring structure, and disulfide linkages.<sup>26</sup>

### 7.6. Cell Targeting Ligand (CTL)

Upon administration, cell-targeting ligands attach to receptor on the surface of the cell with high affinity that is able to go through proficient internalization. A large range of antibodies and polypeptides as potential ligands are available. Delivery of ASOs using different cell-penetrating peptides achieved strong biological impacts at very small concentrations.<sup>27</sup>

### 7.7. Nanocarriers for Oligonucleotide Delivery

ASOs, when delivered with nanoparticle encapsulation, additionally enhance their targeted site of action and improve gene silencing activity. Nanoparticle-based delivery systems to deliver ASOs drugs are achieved by using various polymers, such as, polymeric nanoparticles,<sup>28</sup> polymer micelles,<sup>29</sup> quantum dots,<sup>30</sup> and lipoplexes.<sup>31</sup>

### 7.8. Cationic Polymer Complexes

These cationic amphiphiles containing cholic acid moieties are linked via alkyl-amino side chains. The

cholic acid moieties allow entry through the lipid bilayer, while cationic alkyl-amino chains assist ASOs for binding to its receptor or target site. These amphiphile provides a huge enhancement in targeting the ASO.<sup>32</sup>

## 7.9. Viral Vectors

Antisense sequences encoding into viral vectors with small nuclear RNAs have been built for permitting proper subcellular localization of ASO with pre-mRNAs. Viral vectors, such as, retroviral, adenoviral vectors, dominate clinical gene therapy which addressed excellent delivery approach for ASOs.<sup>33,34</sup>

## 7.10. Polycations

Polycation is a well-known methodology intracellular delivery of therapeutic drugs, which includes the utilization of polyethylene amine complexes and dendrimers. They apply proton-sponge effect, by which the content of endosomes is released into the surrounding.<sup>35,36</sup>

## 7.11. PEGylated Polycations

PEGylated compounds have been developed to sterically protect the delivery vehicles from the undesired effects of blood components. It also overcomes the impacts of serum protein binding and hence, prevents the adverse effects. But, it might also decrease the uptake of ASOs by target cells.<sup>37</sup>

## 7.12. Lipoplexes

Lipoplexes consist of cationic lipids that interact with the anionic lipids of endosome membrane and exert destabilizing effects. This leads to the formation of non-bilayer structures and endosome instability. A study revealed that ASO conveyed by lipoplexes enter the cells by membrane fusion, which represents the functional induction of the target gene.<sup>38</sup>

## 7.13. Bacterial Mini Cells

Recombinant mini cells are being utilized as a targeted drug delivery system. Bacterial mini cells are sphere-shaped cell, which lacks chromosomal DNA and are, thus, unable to proliferate. These mini cells, containing miRNA, siRNA, or plasmid are targeted for delivery to specific cells or tissues via surface expressing antibodies.<sup>39</sup>

## 8. FUTURE PERSPECTIVE

In the course of recent decades, ASO innovation has risen as a substantial way to deal with physiologic and

pathologic procedures. Continuous *in vitro* examinations utilizing ASOs grant the portrayal of new targets and new therapeutic compounds. The delivery and stability issues are right now being effectively addressed, and it is soon likely to turn into a pillar of treatment, revealing insight into approaches to building helpful adequacy and specificity.

## 9. CONCLUSION

Regardless of the present use of ASOs is advancing; still, there are critical issues in utilizing these nucleic acid chains. Development of modified ASOs can now reach specific targeted tissues, such as, brain, placenta, liver, and spleen, and even in gaps in the endothelium, giving hopes for treating various illnesses of humans. Recently, ASOs as therapeutic agents have developed as a substantial approach to deal with specifically modulate gene expression, which has provoked an incredible covenant interest. Lipid and polymer-based nanocarriers still remain significant tools for the delivery of oligonucleotides. A range of ingenious methodologies has dealt with the challenges raised in oligonucleotide delivery. It appears to be likely that huge numbers of the issues in the oligonucleotide therapeutics field can be managed by sensible use of promising delivery strategies that right now exist in developed structures and that are rapidly emerging.

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