

RNase H antisense mechanism

After injection, antisense drugs enter the nucleus and hybridize to the specific sequences of target mRNA, forming a sense-antisense duplex. This duplex leads to initiation of recruitment of Ribonuclease H (RNase H) enzyme, which is an endogenous nuclease. Further, RNase H degrades the target mRNA, inhibiting target mRNA expression. Antisense drug moves on to another target

Figure 1: RNase H Mechanism

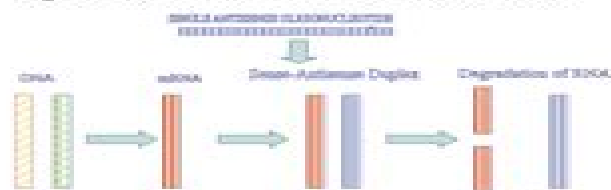


FIGURE 2: siRNA Mechanism



Fig 3 : First generation ASON



mRNA¹¹. (Fig 1)

dsRNA antisense mechanism

dsRNA antisense mechanism is a new technique. It differs from the RNase H antisense mechanism from the initiation step itself, which involves the use of double stranded oligonucleotides. After injection of double stranded antisense drug, the two strands (antisense and sense) separate and RISC complex (an endogenous conglomerate of enzymes) comes to associate with the antisense strand. This antisense strand -RISC complex bind to target mRNA, leading to formation of sense-antisense duplex. Further, RNase III component of RISC complex degrades the target mRNA, thereby inhibiting target mRNA expression¹².

(Fig 2)

SYNTHESIS OF ANTISENSE OLIGONUCLEOTIDES

Naturally occurring antisense oligonucleotides are phosphodiester compounds. Phosphodiester oligonucleotide compounds are easily synthesized, but they

are not used as antisense drugs because they are readily digested by nucleases. To make these compounds more nuclease resistant, modifications at various levels like base, sugar, internucleotide linkages are done¹³. Earliest modification was replacement of one of the oxygen atoms by a methyl group, at each phosphorus in the oligonucleotide chain, producing a methylphosphonate oligonucleotide. But, this compound was found to have reduced antisense activity¹⁴.

FIRST GENERATION OLIGONUCLEOTIDE ANALOGS (TABLE I)

A break through in the research of antisense drugs came with the development of phosphorothioate oligonucleotide compounds. Replacement of one of the oxygen atoms by sulphur in the phosphorus group of oligonucleotide chain gives rise to phosphorothioate oligonucleotide. These compounds are more nuclease resistant and have a longer half-life^{15,16}(Fig 3)

PHARMACOKINETICS

Phosphorothioates are absorbed rapidly after parenteral administration¹⁷. However, other routes of administration are also tried. A trial comparing topical and intravenous administration of alicaforsen in psoriasis patients gave equivocal results, thus indicating that the antisense agent is well absorbed by topical route also¹⁸. Antisense drugs have also been given via aerosols for inhalation through lungs in various trials¹⁹. These compounds are distributed rapidly and extensively. Distribution is to all the tissues, however, liver, kidney and bone marrow accumulate maximum percentage of the drug²⁰. They do not cross the blood brain barrier. Phosphorothioates bind to serum albumin and α-2 macroglobulin. Increased protein binding causes delayed renal clearance and increased distribution in peripheral tissues²¹. Metabolism of phosphorothioates is mediated by exo-nucleases and endo-nucleases resulting in shorter oligonucleotides and nucleosides, which are finally degraded. Elimination occurs via the kidney. The phosphorothioates are filtered by the glomerulus and then undergo tubular reabsorption. They are also found to distribute in the Bowman's capsule, proximal convoluted tubule, brush border membrane and renal tubular epithelial cells. The elimination half-life of drug from renal medulla is 156 hours²².

CELLULAR UPTAKE

It has been seen that phosphorothioates are taken up by a wide variety of cells. Preclinical studies using immunofluorescent and autoradiographic approaches have exhibited the localization of these compounds in a variety of cells in the body, like skin cells, liver cells, renal cells and bone marrow cells²³. Receptor mediated endocytosis is one of the postulated mechanism, about which there is yet inconclusive evidence²⁴.

Some new drug delivery strategies have been developed to enhance cellular uptake. Cationic lipids have been used to increase cellular uptake²⁵. Other approaches include usage of liposomal formulations, dextran sulfate and streptolysin D treatment of cells^{26,27}.

TOXICOLOGY

Limited understanding of the basic mechanism of antisense drugs has raised concern about the genotoxicity and other

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Handbook of Molecular-Genetic Techniques for Brain and Behavior Research Wim E. Crusio, R.T. Gerlai, 1999-10-18 The book gives a broad overview of recombinant DNA techniques for the behavioral neuroscientist with illustrative examples of applications Species covered include rodents mainly mice *Drosophila melanogaster* *Caenorhabditis elegans* and *Danio rerio* Experimental techniques required to characterize the behavioral phenotypes of mutant animals is provided Several aspects of novel molecular genetic techniques are overviewed and possible research strategies are explained The sections of the book start with general descriptions of techniques followed by illustrative examples It is divided into six sections Section 1 bioinformatics and genomics research Section 2 top down strategies where the researcher starts with the phenotype and then analyzes the associated genes bottom up strategies where the physiological chain leading to a phenotype is analyzed starting from the gene product Section 3 transgenic approaches in rodents including overexpressing foreign genes and gene targeting systemic manipulation approaches directly targeting the central nervous system and methods used with invertebrates Section 4 methods used to evaluate relevant behavioral phenotypes including learning and aggression Section 5 examples on molecular brain research in man Section 6 ethical aspects of research in this field

Brain Research, 1998-11 **Danish Medical Bulletin**, 2001 **Netter's Illustrated Pharmacology** Robert B. Raffa, Elena Portyansky Beyzarov, Scott M. Rawls, 2004 Using the classic images of physician artist Frank H Netter as well as other talented artists this book offers a unique visual approach to learning the basic principles of pharmacology Classified by specific organ systems each chapter shows how drugs are used for specific disorders of that system The authors also include clear concise notes under each image highlighting the important elements of each principle

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Staelin Jensen, Judith A. Turner, Zsuzsanna Wiesenfeld-Hallin, 1997 Proceedings of the August 1996 congress with sections on pain in society transition of pain from acute to chronic neurobiology of persistent pain pharmacology of pain neurotrophic factors and pain quantitative measures of pain musculoskeletal pain neuropathic pain psychosocial factors in pain cancer pain new insights into opioid analgesics processing and imaging of pain and pain in infants children and the elderly Specific topics include cultural influences on pain the nociceptive system the pharmacology of new agonists and antagonists in peripheral nociception and cloned opioid receptors Annotation copyrighted by Book News Inc Portland OR **ONR**
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Principles & Practice of Medical Therapy in Pregnancy Louis Buttino, 1998 The encyclopedic coverage includes all medical treatment of pregnant women from general aspects of medical care during pregnancy to critical care and surgery in pregnancy The third has been thoroughly revised and features a new section on medical genetics International Books in Print, 1997 Transmitter Amino Acid Receptors Eric A. Barnard, Erminio Costa, 1991 Neoglycoconjugates Yee Chun Lee, Reiko T. Lee, 1994-05-05 Neoglycoconjugates are important tools in the study of the biological roles of carbohydrates This book provides a survey of synthetic strategies for neoglycoconjugate production and includes applications in medicine particularly in vaccine preparation drug targeting and tumour diagnosis *Symposium on Macromolecules*, 1994

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