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ANTISENSE THERAPEUTICS AND THE TREATMENT OF CNS DISEASE

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1. ABSTRACT

Antisense oligonucleotides (ONs) have great therapeutic potential for conditions in which aberrant protein production results in pathology. This method of reducing the expression of a target gene is both precise and sequence-specific. Although there are many applications for antisense ONs as central nervous system (CNS) therapeutics, systemically administered antisense ONs must be capable of crossing the blood-brain barrier (BBB) in quantities effective enough to alter protein production in the CNS. Because antisense ONs are large, highly polar molecules, their rate of transport across the BBB is likely to be low. Recent studies have shown that antisense ONs are capable of crossing the BBB without the aid of a carrier system, however little is known about the molecular mechanisms which mediate this transport. This review will focus on nucleic acid chemistries suitable for *in vivo* research and their potential applications in the treatment of CNS disease.

2. INTRODUCTION

Antisense oligonucleotides (AS ONs) are tools which can be used to alter the expression of target proteins. Their mechanism of action is simple yet highly specific. Expression of genomic DNA relies on its transcription into mRNA which is then translated into protein. An antisense ON is a short nucleic acid which hybridizes to its complementary mRNA by the formation of Watson-Crick hydrogen bonds, thus preventing its translation into protein. Translation can be prevented by one or both of two possible mechanisms. The first involves activation of RNase H. RNase H is a naturally occurring enzyme which, when activated, will cleave the RNA moiety in a DNA:RNA heteroduplex leading to degradation of the target mRNA (1, 21). Antisense ONs which do not stimulate RNase H activity can down-regulate protein expression by sterically blocking the translation machinery from binding to their mRNA target. ONs that hybridize with double-helical DNA by forming Hoogsten or reverse-Hoogsten hydrogen bonds can be used to target the actual gene of interest. This technique is known as the antigene or triple helix strategy. For a review of current antigene strategies see reference (22). The present review, however, will focus primarily on antisense technology and its potential therapeutic applications in central nervous system (CNS) disease.

The CNS is a complex network which is essential to our ability to function in the everyday world. Although some degree of flexibility is built into this system, the complexity of the CNS creates potential for disease when small abnormalities arise. Genetic mutations leading to aberrant gene expression are often linked to CNS dysfunction and, as our understanding of the CNS improves, effective therapeutic targets are slowly being discovered. Although sequencing of the human genome has revealed many genetic mutations which have the potential for CNS malfunction, this list is far from exhaustive. AS ONs are a powerful tool with the potential to address questions about the function of genes expressed in the CNS. In theory, these molecules could be used to treat any disease caused by expression of genes which encode for deleterious proteins. However, because entry into the CNS is restricted by both the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB), systemically administered AS ONs must be capable of overcoming

these obstacles to reach their target and achieve full therapeutic potential.

3. WHAT IS THE BLOOD-BRAIN BARRIER?

3.1. The Blood-Brain Barrier

The BBB is an important means of protecting the central nervous system (CNS) from blood-borne substances including foreign substances such as viruses or toxins. Unlike peripheral capillaries, the capillaries which deliver nutrients to the brain parenchyma are comprised of endothelial cells bound together by tight junctions. These tight junctions limit the exchange of materials between the general circulation and the CNS. Mechanisms must exist to promote the movement of substances such as oxygen and nutrients into and out of the brain. Solutes can interact with the BBB in a variety of ways to traverse the endothelium. Often, the mechanism used to move across the BBB requires certain physical characteristics to be present within the molecule. For example, some solutes can passively diffuse across the endothelial cells by dissolving into the lipids within the cell membrane. This non-saturable mechanism of transport is inversely correlated with the square root of the molecular weight and directly correlated with lipophilicity/hydrogen bonding (10, 12, 34). Lipid solubility can be determined experimentally by calculating the fraction of the substance which partitions into the two layers of an aqueous/octanol mixture. Hydrophobic substances, such as antipyrine, demonstrate a large partition coefficient and are readily absorbed across the endothelial cells of the BBB. Molecules which are hydrophilic, however, do not passively diffuse well across the BBB. These molecules are associated with a small partition coefficient and limited brain uptake unless a saturable transport mechanism exists.

Molecules that are large or hydrophilic are sometimes transported across the BBB by carrier-mediated systems. Carrier-mediated transport across the BBB is saturable because the rate of uptake is dependent on a limited number of transporters and, when excess ligand is present, these transporters reach capacity. Carrier-mediated transport mechanisms ensure that the brain receives an adequate supply of nutrients, since molecules essential for brain function are often too large to cross the BBB by passive diffusion. For example, the GLUT-1 transporter which is present in the endothelial cells of the BBB is responsible for the transport of glucose from the systemic circulation into the brain (24, 35). Various saturable transport systems also exist for the transport of amino acids into the CNS (11, 12).

3.2. The Blood-Cerebrospinal Fluid Barrier

While the tightly joined endothelial cells of the cerebral capillaries form the barrier between the brain parenchyma and the blood, a single layer of specialized epithelial cells comprise the interface between the blood and the cerebrospinal fluid (CSF). This layer of simple cuboidal epithelial cells, joined together by tight junctions, is collectively known as the choroid plexus (CP). The cells of the CP are polarized with the apical side facing the CSF and the basolateral side oriented towards a core of loose connective tissue and cells of lymphoid lineage. Embedded in this core region are large fenestrated capillaries. Unlike the capillaries of the BBB, these capillaries are highly permeable, so it is the CP which must provide the barrier function to protect the CSF from harmful substances.

Although the CP is less thoroughly studied than the vascular BBB, the blood-CSF interface is actually a large area which plays a significant role in blood-CNS exchange. In fact, the surface area of the apical region of the CP is comparable to the surface area of the luminal region of the endothelial cells which comprise the BBB (26). It is possible that systemically administered drugs which are not transported across the BBB could gain direct access to the CNS by crossing at the CP. For example, the CP appears to be slightly more permeable to polar compounds than the BBB. Stavudine (D4T) is a hydrophilic drug which cannot cross the BBB; however, previous studies have shown significant concentrations can diffuse across the CP into the CSF (43).

Current studies have shown that certain modified nucleic acid analogs can cross the BBB without the use of a delivery vehicle. For example, peptide nucleic acids (PNAs) directed against the neurotensin receptor (NTR1), the dopamine transporter and the mu opiate receptor in rats were all found to cross the BBB in quantities sufficient to elicit a behavioral response (30, 44, 45). Another modified antisense ON with the potential to cross the BBB is a phosphorothioate oligodeoxynucleotide (PS-ODN) directed at the Amyloid ß (Aß) protein. Systemic administration of this antisense ON resulted not only in an altered behavioral response, but also significant down-regulation of protein expression (3).

Although these studies indicate that systemically administered modified nucleic acid analogs can enter the CNS, it is still unclear which specific chemical modifications allow for transport across the BBB and whether these modifications allow for entry at the blood-CSF barrier as well. Many chemical alterations have been used to create novel nucleic acid analogs with desirable characteristics such as increased stability in biological fluids and higher affinity for the nucleic acid target, however few studies have explored how these changes could affect entry into the CNS. A few common nucleic acid chemistries are discussed below along with their unique pharmacokinetic properties.

4. ANTISENSE OLIGONUCLEOTIDE MODIFICATIONS

Previous studies have shown that unmodified oligonucleotides are degraded rapidly in biological fluids by nucleases. For this reason, chemical modifications are often introduced to increase stability. Modifications include base-modification, modified ribose sugars (usually at the 2' position) or alteration of the phosphate backbone. Very few analogs with unnatural bases have been studied *in vivo* so the main focus of this section will be on backbone and sugar modifications.

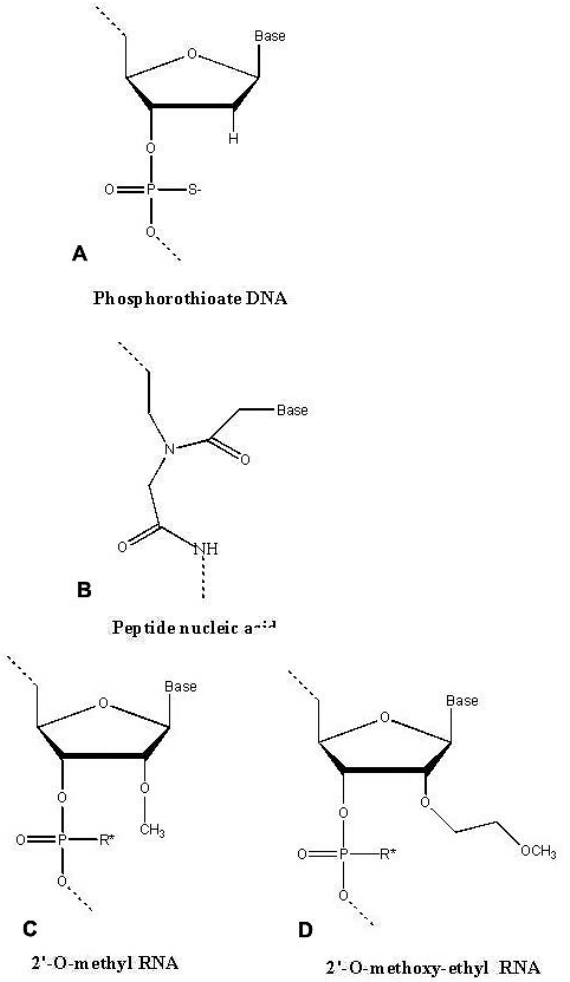

Figure 1. Chemical structures of antisense ONs discussed in this review.

4.1. Phosphorothioate Oligonucleotides

The phosphorothioate (PS) modification (Figure 1A) replaces a non-bridging oxygen atom in the phosphate backbone with a sulfur. This modification enhances resistance to nuclease degradation. Upon binding to the target mRNA, PS-ODNs form a DNA-RNA heteroduplex which activates an enzyme called RNase H. This enzyme cleaves the RNA moiety of the heteroduplex, thus degrading the target mRNA so translation into protein does not occur. Like DNA, PS-ODNs carry a negative charge at physiological pH, therefore they are highly soluble in water. However, this property also serves to enhance non-specific interactions with plasma proteins such as albumin and alpha-2 macroglobulin (19). This binding to plasma proteins offers protection from filtration and thus increases the serum half-life of PS-ODNs. However, non-specific protein interactions may also be responsible for the toxicity associated with high doses of PS-ODNs. Rapid clearance from plasma is primarily due to the initial rapid distribution of PS-ODNs into peripheral organs with liver and kidney accumulating the highest concentration of oligonucleotide (7). Conversely, tissue clearance is relatively slow, thus creating reservoirs from which PS-ODNs are continuously released. The accumulation of PS-ODNs in tissues is likely caused by an interaction between the ODN and an intracellular protein.

4.2. Peptide Nucleic Acids

A peptide nucleic acid (PNA) (Figure 1B) is a nucleic acid mimic in which the sugar-phosphate backbone is replaced by an uncharged backbone made of amide linkages, like those found in peptides. The bases are attached by methylene carbonyl linkers. PNAs demonstrate high stability in biological fluids, a characteristic which is probably due to their unique chemical structure. An amide bond connecting two natural amino acids is a motif not found in nature; therefore, many proteolytic enzymes do not recognize PNAs as a substrate. For example, studies have shown that PNAs are resistant to degradation by nucleases (including RNase H), proteases and peptidases (14). Although PNA/RNA heteroduplexes are not substrates for the RNase H enzyme, PNAs are still able to mediate antisense effects by sterically blocking the translation machinery from access to the target mRNA. Because of this feature, as the stability of the PNA/RNA hybrid increases, the antisense effect of the PNA will become more efficient.

PNAs are electrostatically neutral molecules, therefore they have a low affinity for proteins which usually bind charged nucleic acids. Unlike PS-ODNs, PNAs are cleared very rapidly from the plasma by glomerular filtration (6, 36). Modification which removes the charge from the backbone seems to decrease non-specific interactions with proteins but may also affect tissue distribution of the PNA, thus decreasing its half-life in the body. PNAs can bind complementary single stranded RNA or DNA to form a duplex, however PNAs can also hybridize with double stranded DNA to induce triplex formation. The thermodynamic stability of PNA/nucleic acid duplexes is higher than that of PS-ODNs. Previous studies have shown that it often exceeds that of corresponding DNA/RNA and DNA/DNA duplexes (15, 33). This property is probably due to the fact that the PNA backbone is uncharged, therefore PNA/nucleic acid duplexes lack the electrostatic repulsion usually seen between DNA/DNA or DNA/RNA duplexes.

4.3. Alkyl Modifications

Antisense oligoribonucleotides (AS-ORNs) which contain alkyl modifications at the 2’ position of the ribose sugar have also been developed (Figure 1C and D). The two most common modifications of this type include the 2’-O-methyl (2’-OMe) and the 2’-methoxyethyl (2’-MOE) modifications to RNA. Compared to PS-ODNs, AS-ORNs containing this type of modification demonstrate enhanced stability in biological fluids (due to nuclease resistance), and increased affinity for their complementary RNAs (17). Like PNAs, 2’-O-alkyl RNAs cannot activate RNase H, therefore their antisense effect is probably due to a steric block of translation or an RNase H independent mechanism. AS-ORNs containing these modifications are less toxic than PS-ODNs, possibly due to their low binding affinity for plasma proteins. A recent study suggests that the introduction of MOE groups on the 2’-ribose sugar

increased permeability across the rat intestine as compared with a matched PS-ODN containing the same nucleotide sequence (27).

5. ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is characterized by the formation of aggregates of a fragment of the amyloid precursor protein (APP). APP is a type I transmembrane protein which can be cleaved into toxic fragments. It contains three specific cleavage sites targeted by the a-,ß-, and ?-secretase enzymes, respectively. When full-length APP is cut at the a-secretase cleavage site, the resulting fragments are not harmful to the cell. However, when cleaved at both the ß- and ?-secretase sites, a fragment which can fold into a self-aggregating shape is formed. Because the cleavage site for the ?- secretase is variable, this peptide fragment can be between 39 to 43 amino acids long. The most toxic fragment is known as Amyloid ß (Aß) $_{1-42}$, however the $A\beta_{1-40}$ fragment is produced with greater frequency (39). While $A\beta_{1-40}$ is the predominant form of cerebrovascular amyloid, $A\beta_{1-42}$ is the major constituent of senile plaques (18, 28, 31, 37). These deposits are found in the extracellular spaces of the brain and are associated with the death of the neurons that surround them.

Antisense ONs could be ideal candidates for the treatment of AD and other related forms of cerebrovascular amyloidosis. If levels of Aß in the brain could be reduced, then the onset of the disease could be slowed or possibly prevented altogether. Possible antisense targets for treatment of the disease include the mRNA for Aß or for the ß- and ?-secretases. However, because peripherally administered drugs designed to treat AD must reach the brain to produce effective results, the BBB presents a potential obstacle which can prevent delivery of therapeutic antisense ONs. Two recent studies have shown that two different types of antisense ONs, a peptide nucleic acid (PNA) and a P-ODN, can successfully cross the BBB and reduce levels of Aß in mice (3, 29).

In the study by Banks *et al.* (3), a P-ODN was directed at the nucleotides coding for amino acids 17-30 in the Aß region of the APP gene. The P-ODN was radioactively labeled with ^{32}P and administered by i.v. injection. Radioactivity was measured in both the parenchymal space of the brain and also in the cerebrospinal fluid. SDS-PAGE (polyacrylamide gel electrophoresis) confirmed that the P-ODN was transported intact across the BBB. The presence of a saturable transport system, which was named oligonucleotide transport system-1 (OTS-1), was confirmed when brain uptake of radioactively labeled P-ODN was found to be inhibited by the presence of unlabeled Aß P-ODN. Administration of the P-ODN by tail vein injection reversed learning and memory deficits in 12 month old SAM-P8 mice. At four months of age, the mice appear phenotypically normal, however at 12 months of age the mice have developed severe deficits in learning and memory. This strain of mouse was chosen because it has been found to overexpress APP as it ages due to a spontaneous mutation. Previous work had shown that antisense directed at various regions of the APP gene could alleviate learning and memory deficits in the 12 month old SAM-P8 mouse when administered directly into the brain by intracerebroventricular injection.

In a study by McMahon *et al.* (29), eight PNA sequences were administered to mice by either a single intraperitoneal (i.p.) injection or four days of daily i.p. injections. A PNA directed at a region six bases upstream of the initiation site on the APP gene was the only sequence detected in the mouse brain by gel-shift assay. The PNA was not detected after the single i.p. injection, however it was detectable after the four daily injections. Although this study implies that PNAs administered systemically can cross the BBB, at this time the mechanism of entry remains unknown.

Although antisense pharmacotherapy is a promising treatment for AD, it is not without problems. For example, it is important to note that some APP must be present in the brain to carry out normal functions, such as iron transport (46). $A\beta_{1-40,}$ an APP cleavage product, also has a role in neuroprotection. Studies have shown that small concentrations of this molecule are necessary to maintain healthy neurons in culture (47). Therefore, complete elimination of APP and its cleavage products for extended periods of time may be detrimental to humans. In conclusion, successful treatment of AD using antisense pharmacotherapy would involve adjusting the dosage of antisense to accommodate for the levels of APP necessary to maintain healthy brain functioning while also keeping protein concentrations below the levels which promote neurotoxicity.

6. CANCER

Because cancer is often caused by abnormal expression of proteins, it would be another pathological condition in which antisense therapeutics would be useful. Potential antisense targets for cancer therapy include proteins involved in mitosis, such as protein kinase C (PKC), or regulators of cell cycle, such as the cyclin dependent kinases (CDKs). Slowing cellular replication is not the only mechanism by which antisense could be used for cancer therapy. Mutations in the genes encoding for apoptotic proteins, such as B-cell lymphoma protein- 2 (Bcl-2) and myeloid cell leukemia protein- 1 (Mcl-1), are also associated with human malignancy.

Apoptosis is an irreversible mechanism used to safely eliminate damaged cells. A family of enzymes called caspases are the effector molecules of this programmed cell death. These cysteine proteases cleave specific intracellular substrates leading to the decay of affected cells. The Bcl-2 family of proteins acts upstream of these enzymes to provide a regulatory checkpoint. The members of this family share structural homology, however their function can be either pro-apoptotic (i.e. Bax) or antiapoptotic (i.e. Bcl-2 and Mcl-1). Chemotherapeutic agents often modulate apoptotic pathways to induce cytotoxicity of tumor cells; however, over-expression of the anti-apoptotic Bcl-2 family of proteins contributes to the eventual resistance to

treatment (25, 38, 41). Antisense therapy directed against one or more of the antiapoptotic proteins of the Bcl-2 family could be a promising approach to overcome chemoresistance.

In a recent study by Thallinger *et al.* a 2'-O-methoxy-ethyl/2'-deoxynucleotide chimeric phosphorothioate antisense oligonucleotide directed against Mcl-1 was used to downregulate Mcl-1 protein expression in human melanoma cells both *in vitro* and *in vivo.* Furthermore, treatment with the antisense directed against Mcl-1 enhanced chemosensitivity of human tumor cells to dacarbazine, an antineoplastic agent, in a severe combined immunodeficient (SCID) mouse xenotransplantation model. Expression of Bcl-2 and Bcl-xl, two proteins which are structurally homologous with Mcl-1, was not altered by antisense treatment with anti-Mcl-1 oligonucleotide (42). This indicates that the effects of antisense treatment on protein expression were highly specific.

Another protein associated with chemoresistance is P-glycoprotein (P-gp). P-gp is a transmembrane "pump" which is responsible for efflux of drugs and other toxins from cells. P-gp is encoded by the *mdr*1 gene. Overexpression of this gene can decrease response to chemotherapeutic agents. Because inhibition of P-gp is highly toxic, a better method of managing drug resistance would be through the modulation of *mdr*1 gene expression. Previous studies have examined the effects of PS-ODNs directed against *mdr*1 (2, 5) however, because the gene is overexpressed, the concentration of PS-ODN used is very large. At high concentrations, PS-ODNs can cause non-antisense effects due to their non-specific protein binding (40). RNase H inhibition can also occur at high concentrations (13).

In a recent study by Brigui *et al.* (8), modifications were introduced into a PS-ODN previously shown to affect *mdr*1 expression. This antisense ODN was termed a "minimally modified" PS-ODN. At the 3'-end, it contained a self-forming hairpin to protect it against 3'-exonucleases, while at the 5'-end 2 phosphorothioate groups were introduced to protect the ODN from 5'-exonucleases. The internal phosphorothioate linkages were absent. Internalization of antisense into cells was achieved with either addition of a commercially available cholesterol group called Superfect or an amphotericin B cationic derivative. Although the minimally modified PS-ODN had increased nuclease resistance, the ODN composed of all phosphorothioate linkages had a significantly greater antisense effect.

7. HIV

Under normal conditions, microbial invasion of the CNS is prevented by the physical barrier created by the BBB and the CSF-brain interface. However, HIV-1 enters the CNS early during the course of infection. The virus is thought to enter the brain through the infiltration of infected immune cells or as free virus, although the exact mechanisms of entry are still incompletely understood. The BBB and blood-CSF barrier prevent drug concentrations from reaching therapeutic levels in the CNS. Lower drug concentrations in the brain may allow for a site of continuous viral replication. In this manner, the brain acts as a potential viral reservoir, sequestering HIV-1 virons which could be re-released into the general circulation to reinfect peripheral tissues. Possible mechanisms for viral re-entry into the circulation include saturable efflux across the brain microvasculature or venous reabsorption of CSF at the arachnoid villi (blood-CSF barrier). In a study by Cashion *et al.* (9), radioactively labeled gp120 was administered to mice by i.c.v. injection. This viral coat protein was able to exit the brain through the brain's primitive lymphatic system. Through drainage of brain CSF into the cervical lymph nodes, HIV-1 virons could potentially reinfect lymphoid tissue without exposure to anti-viral drugs that may be circulating in the blood.

Binding of glycoprotein (gp) 120 to both CD4 and a chemokine coreceptor initiates viral entry into CD4+ cell types. CD4 is typically found on T lymphocytes, blood monocytes, macrophages and some dendritic cells. Adult brain endothelial cells, however, do not express CD4 (32). Therefore, the receptor mediating entry of cell-free virus into the brain microvasculature is still unclear. In mice, cell-free HIV-1 pseudoviruses were found to cross the BBB after i.v. administration by an event related to adsorptive endocytosis (4). Envelope proteins, such as gp120, appear to be necessary for the transport of these virons into the CNS. Virus taken up from the circulation appeared in both the brain parenchyma and the CSF, indicating that HIV-1 can enter the CNS from both the brain microvasculature and also at the choroid plexus.

Transcription of viral DNA is regulated by the trans-activating response element (TAR) (23). Tat, another virally encoded protein, interacts with TAR to recruit TAK, a host cell protein kinase complex which consists of cyclin dependent kinase 9 (Cdk 9) and Cyclin T (Cyc T) (16). The TAR-Tat-TAK interaction is essential for viral mRNA synthesis. Disruption of this complex can impair viral replication. In a recent study by Hamma et. al (20), an oligo-2'-O-methylribonucleoside with an alternating methylphosphonate/ phosphodiester backbone (mr-AOMP) directed against the TAR apical stem loop, was used to inhibit the formation of the Tat-TAR complex. The anti-TAR oligomer used in this study was found to prevent the Tat-TAR interaction *in vitro* as well as inhibit Tat-mediated transcription of a chloramphenicol acetyl transferase (CAT) reporter gene in a concentration-dependent manner. These results indicate that disruption of the Tat-TAR interaction with an antisense oligomer is a method which could be used to inhibit HIV replication.

8. CONCLUSION

Recent studies have shown that systemically administered antisense ONs are capable of crossing the BBB in concentrations effective to alter the expression of proteins in the brain; however, little is known of the molecular mechanisms which mediate this type of transport. Because antisense technology holds great potential for the treatment of CNS pathology, it is imperative that future research focuses on providing a better

understanding of the degree of antisense ON transport at both the blood-brain and blood-CSF interfaces. The characteristics of antisense ONs which allow them to cross into the CNS should also be considered since the type of modification used as well as the degree to which the modification was used can cause drastically different pharmacokinetic profiles.

9. REFERENCES

1. Agrawal, S., Z. Jiang, Q. Zhao, D. Shaw, Q. Cai, A. Roskey, L. Channavajjala, C. Saxinger and R. Zhang: Mixed-backbone oligonucleotides as second generation antisense oligonucleotides: *in vitro* and *in vivo* studies. *Proc Natl Acad Sci* USA 94, 2620-2625 (1997)

2. Alahari, S. K., N. M. Dean, M. H. Fisher, R. Delong, M. Manoharan, K.L. Tivel and R.L. Juliano: Inhibition of expression of the multidrug resistance-associated P-glycoprotein by phosphorothioate and 5' cholesterol-conjugated phosphorothioate antisense oligonucleotides. *Mol Pharmacol* 50, 808-819 (1996)

3. Banks, W. A., S. A. Farr, W. Butt, V. B. Kumar, M. W. Franko and J. E. Morley: Delivery across the blood-brain barrier of antisense directed against amyloid β: reversal of learning and memory deficits in mice overexpressing amyloid precursor protein. *J Pharmacol Exp Ther* 297, 1113-1121 (2001)

4. Banks, W. A., E. O. Freed, K. A. Wolf, S. M. Robinson, M. Franko and V. B. Kumar: Transport of human immunodeficiency virus type 1 pseudoviruses across the blood-brain barrier: role of envelope proteins and adsorptive endocytosis. *J Virol* 75, 4681-4691 (2001)

5. Bertram, J., K. Palfner, M. Killian, W. Brysch, K. H. Schlingensiepen, W. Hiddemann and M. Kneba: Reversal of multidrug resistance *in vitro* by phosphorothioate oligonucleotides and ribozymes. *Anticancer Drugs* 6, 124-134 (1995)

6. Boado, R. J. Antisense drug delivery through the blood-brain barrier. *Adv Drug Del Rev* 15, 73-107 (1995)

7. Boado, R. J., Y. S. Kang, D. Wu and W. M. Pardridge: Rapid plasma clearance and metabolism *in vivo* of a phosphorothioate oligodeoxynucleotide with a single, internal phosphodiester bond. *Drug Metab Dispos* 23, 1297-1300 (1995)

8. Brigui, I., T. Djavanbakht-Samani, B. Jollès, S. Pigaglio and A. Laigle: Minimally modified phosphodiester antisense oligodeoxyribonucleotide directed against the multidrug resistance gene mdr1. *Biochem Pharm* 65, 747-754 (2003)

9. Cashion, M. F., W. A. Banks, K. L. Bost and A. J. Kastin: Transmission routes of HIV-1 gp120 from brain to lymphoid tissues. *Brain Res* 822, 26-33 (1999)

10. Chikhale, E. G., K. Y. Ng, P. S. Burton and R. T. Borchardt: Hydrogen bonding potential as a determinant of the *in vitro* and *in situ* blood-brain barrier permeability of peptides. *Pharm Res* 11, 412-419 (1994)

11. Christensen, H. N: Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev* 70, 43-77 (1990)

12. Cornford, E. M: The blood-brain barrier, a dynamic regulatory interface. *Mol Physiol* 7, 219-260 (1985)

13. Crooke, S. T: Therapeutic applications of oligonucleotides. *Annu Rev Pharmacol Toxicol* 32, 329-376 (1992)

14. Demidov, V. V., V. N. Potaman, M. D. Frank-Kamenetskii, M. Egholm, O. Buchard, S. H. Sonnichsen and P. E. Nielsen: Stability of peptide nucleic acids in human serum and cellular extracts. *Biochem Pharm* 48, 1310-1313 (1994)

15. Egholm, M., O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden and P. E. Nielsen: PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen bonding rules. *Nature* 365, 566-568 (1993)

16. Emerman, M. and M. H. Malim: HIV-1 Regulatory/Accessory Genes: Keys to Unraveling Viral and Host Cell Biology. *Science* 280, 1880-1884 (1998)

17. Geary, R. S., T. A. L. Truong Watanabe, S. M. E. A. Lesnik Freier, N. B. H. Sasmor Sioufi, M. Manoharan, A. A. Levin: Pharmokinetic properties of 2'-O-(-2-methoxyethyl)-modified oligonucleotide analogues in rats. *J Pharmacol Exp Ther* 296, 890-897 (2001)

18. Glenner, G. G., C. W. Wong: Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem and Biophys Res Comm* 120, 885-890 (1994)

19. Greig, M. J., H. Gaus, L. L. Cummins, H. Sasmor and R. H. Griffey: Measurement of macromolecular binding using electrospray mass spectrometry. Determination of disassociation constants for oligonucleotide:serum albumin complexes. *J Am Chem Soc* 117, 10765-10766 (1995)

20. Hamma, T., A. Saleh, I. Huq, T. M. Rana and P. S. Miller: Inhibition of HIV Tat-TAR interactions by an antisense oligo-2'-O-methylribonucleoside methylphosphonate. *Bioorg Med Chem Lett* 13, 1845-1848 (2003)

21. Hausen, P., H. Stein and H. Ribonuclease: Enzyme degrading the RNA moiety of DNA/RNA hybrids. *Eur J Biochem* 14, 278-283 (1970)

22. Herdewijn, P: Heterocyclic modifications of oligonucleotides and antisense technology.

Antisense and Nucleic Acid Drug Dev 10, 297-310 (2000)

23. Jones, K. A. and B. M. Peterlin: Control of RNA initiation and elongation at the HIV-1 promoter. *Annu Rev Biochem* 63, 717-743 (1994)

24. Kalaria, R. N., S. A. Gravina, J. W. Schmidley, G. Perry and S. I. Harik: The glucose transporter of the human brain and blood-brain barrier. *Ann Neurol* 24, 757-764 (1988)

25. Kaufmann, S. H., J. E. Karp, P. A. Svingen, S. Krajewski, P. J. Burke, S. D. Gore and J. C. Reed: Elevated expression of the apoptotic regulator Mcl-1at the time of leukemic relapse. *Blood* 91, 991-1000 (1998)

26. Keep, R. F. and H. C. Jones: A morphometric study on the development of the lateral ventricle choroid plexus, choroid plexus capillaries and ventricular ependyma in the rat. *Brain Res Dev Brain Res* 56, 47-53 (1990)

27. Khatsenko, O., R. Morgan, L. Truong, C. York-Defalco, H. Sasmor, B. Conklin and R. S. Geary: Absorption of antisense oligonucleotides in rat intestine: effect of chemistry and length. *Antisense and Nucleic Acid Drug Dev* 10, 35-44 (2000)

28. Masters, C. L., G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald and K. Beyreuther: Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci* USA 82, 4245-4249 (1985)

29. McMahon, B. M., J. Stewart, A. Fauq, S. Younkin, L. Younkin and E. Richelson: Using peptide nucleic acids as gene-expression modifiers to reduce ß-amyloid levels. *J Mol Neurosci* 19, 71-76 (2002)

30. McMahon, B. M., J. A. Stewart, J. Jackson, A. Fauq, D. J. McCormick and E. Richelson: Intraperitoneal injection of antisense peptide nucleic acids targeted to the mu receptor decreases response to morphine and receptor protein levels in rat brain. *Brain Res* 904, 345-349 (2001)

31. Miller, D. L., I. A. Papayannopoulos, J. Styles, S. A. Bobin, Y. Y. Lin, K. Biemann and K. Iqbal: Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. *Arch Biochem Biophys* 301, 41-52 (1993)

32. Moses, A. V., F. E. Bloom, C. D. Pauza and J. A. Nelson: Human immunodeficiency virus infection of human brain capillary endothelial cells occurs via a CD4/galactosylceramide-independent mechanism. *Proc Natl Acad Sci USA* 90, 10474-10478 (1993)

33. Nielsen, P. E., M. Egholm, R. H. Berg and O. Buchardt: Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. *Science* 254, 1497-1500 (1991)

34. Oldendorf, W. H: Lipid-solubility and drug penetration of the blood-brain barrier. *Proc Soc Exp Biol Med* 147, 813-816 (1973)

35. Pardridge, W. M., R. J. Boado and C. R. Farrell: Brain-type glucose transporter (GLUT-1) is selectively localized to the blood-brain barrier. Studies with quantitative western blotting and in situ hybridization. *J Biol Chem* 265, 18035-18040 (1990)

36. Pardridge, W. M., R. J. Boado and Y. S. Kang: Vector-mediated delivery of a polyamide ("peptide") nucleic acid analogue through the blood-brain barrier *in vivo. Proc Natl Acad Sci USA* 92, 5592-5596 (1995)

37. Prelli, F., E. M. Castaño, G. G. Glenner and B. Frangione: Differences between vascular and plaque core amyloid in Alzheimer's disease. *J Neurochem* 51, 648-651 (1988)

38. Reed, J. C: Bcl-2: prevention of apoptosis as a mechanism of drug resistance. *Hematol Oncol Clin North Am* 9, 451-473 (1995)

39. Shoji, M., T. E. Golde, J. Ghiso, T. T. Cheung, S. Estus, L. M. Shaffer, X. D. Cai, D. M. McKay, R. Tintner and Frangione, B: Production of the Alzheimer amyloid-ß protein by normal proteolytic processing. *Science* 258, 1598-1603 (1992)

40. Stein, C. A. and Y. C. Cheng: Antisense oligonucleotides as therapeutic agents: is the bullet really magical? *Science* 261, 1004-1012 (1993)

41. Strasser, A., D. C. Huan and, D. L. Vaux: The role of the bcl-2/ced-9 gene family in cancer and general implications of defects in cell death control for tumourigenesis and resistance to chemotherapy. *Biochim Biophys Acta* 1333, F151-F178 (1997)

42. Thallinger, C., M. F. Wolschek, V. Wacheck, H. Maierhofer, P. Günsberg, P. Polterauer, H. Pehamberger, B. P. Monia, E. Selzer, K. Wolff and B. Jansen: Mcl-1 antisense therapy chemosensitizes human melanoma in a SCID mouse xenotransplantation model. *J Invest Dermatol* 120, 1081-1086 (2003)

43. Thomas, S. A. and M. B. Segal: The transport of the anti-HIV drug, 2',3'-didehydro-3'-deoxythymidine (D4T), across the blood-brain and blood-cerebrospinal fluid barriers. *Br J Pharmacol* 125, 49-54 (1998)

44. Tyler-McMahon, B. M., J. A. Stewart, J. Jackson, M. D. Bitner, A. Fauq, D. J. McCormick and E. Richelson: Altering behavioral responses and dopamine transporter protein with antisense peptide nucleic acids. *Biochem Pharm* 62, 929-932 (2001)

45. Tyler, B.M., K. Jansen, D.J. McCormick, C.L. Douglas, M. Boules, J.A. Stewart, L. Zhao, B. Lacy, B. Cusack, A.

Fauq and E. Richelson: Peptide nucleic acids targeted to the neurotensin receptor and administered i.p. cross the blood-brain barrier and specifically reduce gene expression. *Proc Natl Acad Sci USA* 96, 7053-7058 (1999)

46. White, A.R., R. Reyes, J.F. Mercer, J. Camakaris, H. Zheng, A.I. Bush, G. Multhaup, K. Beyreuther, C.L. Masters, R. Cappai: Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice. *Brain Res* 842, 439-444 (1999)

47. Zou, K., J. S. Gong, K. Yanagisawa amd M. Michikawa: A novel function of monomeric amyloid beta-protein serving as an antioxidant molecule against metal-induced oxidative damage. *J Neurosci* 22, 4833-4841 (2002)

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