Biotechnological Aspects of the Antisense Neuropharmaceuticals

ROXANA-GEORGIANA TAUSER[*], ORTANSA STOICA[**]
University of Medicine and Pharmacy "Gr.T.Popa" Iasi,
[*]Faculty of Pharmacy, Chemical Pharmaceutical Dept.,
***Faculty of Medicine, Human Genetic Dept.,*
16 Universitatii street, 6600 Iasi, Romania

Abstract

Antisense oligonucleotides (OGN), either neurotherapeutics or neurodiagnostics, must be conjugated to a brain targeting system in order to hybridize to complementary mRNA and specifically inhibit mutant gene expression in vivo. Chimeric peptide technology as neuropharmaceutical antisense delivery strategy to the brain, supposes the antisense OGN attachment to fusion proteins, which are transported from blood into the brain by absorbtive- or receptor-mediated transcytosis. The main biotechnological aspects regarding antisense neuropharmaceuticals chimeric peptides are discussed.

Keywords: antisense oligonucleotides, antisense neurotherapeutics, antisense neurodiagnostics, brain delivery, chimeric peptide nucleic acids

Introduction

The ***antisense strategy*** consists of specific inhibition of the mutant gene expression *in vivo*, at the level of transcription or translation, by using antisense oligonucleotide directed against DNA or RNA, respectively [1].

Antisense oligonucleotides (antisense OGN) are short, single-stranded, synthetic sequences of chemically modified nucleic acids, which specifically hybridize, according to the base-pairing rules (adenine-tymine/uracil, guanine-cytosine), with the complementary nucleotide sequences from target-DNA or target-RNA, thus blocking the protein synthesis encoded by these targets [2].

Antisense agents could act as *neurotherapeutics* by binding to a specific target mRNA and eliminating the production of that gene product, or as *radiopharmaceuticals* for imaging mutant gene expression in the brain *in vivo*.

Neuropharmaceutical antisense oligonucleotides

The antisense oligonucleotides as neuropharmaceuticals have the following ***advantages*** over the classical antagonists: remarkable selectivity, because antisense OGN hybridize and block the expression of a single subunit of neurotransmitter receptor*;* the possibility of targeting antisense oligonucleotides to specific brain regions responsible for particular pathologic state; the ease design based on the structure of the mRNA encoding the neurotransmitter receptor; the simple synthesis at low costs.

The antisense effect, being preferentially exerted against the pool of the functional neurotransmitter receptors, avoids the compensatory upregulation of the receptors induced by persistent blockade of traditional pharmacological antagonists [3,4,5].

Another advantage of the antisense neuropharmaceuticals consists of the shorter period required to develop an antisense drug, due to powerful *in vitro* and *in vivo* screening methods used to select the most susceptible situs of the targeted mRNA for antisense hybridization and to choose the most specific, selective and stable antisense oligonucleotide from a wide range of chemical modifications.

The ***antisense neurotherapeutic candidates*** might be indicated [3,6] in: *neurodegenerative disorders*, *brain viral disease* and *cerebral tumors*. For instance, in Huntington disease, the molecular target for antisense strategy is either huntingtine transcript or glyceraldehid-phosphat dehidrogenase (GAPDH) mRNA. Other therapeutic uses might be represented by cerebral AIDS because the antisense OGN specific to HIV mRNA, coupled with CNS-targeting systems, could inhibit HIV-1 replication in the brain. In the case of *malignant gliomas*, antisense OGN are directed against aberrant transcripts responsible for the uncontrolled growth and proliferation of the cancer cells.

The neuroprotective role of the antisense oligodeoxynucleotides anti-NMDA-R1 receptor transcript was demonstrated in rats model of cerebral ischemia by: significantly reduction of the focal ischemic infarctions volume, diminishing astrocyte activation and behavioral recovery improvement at 2 weeks after the neurotoxic event [7,8].

In familial Alzheimer disease, phosphorothioate (PS-ODN) antisense directed to presenilin-1 mRNA proved neuroprotective activity against NMDA-induced neuronal cell death, because NMDA-neurotoxicity is associated with high expression levels of presenilin-1.

Antisense radiopharmaceuticals as neurodiagnostic tools are useful for *in vivo* direct imaging mutant gene expression encoding pathologic proteins in the human brain, such as A$\square^{1\text{-}40}$ amyloid deposition in Alzheimer disease [6].

Brain delivery of the antisense oligonucleotides

Antisense OGN are hydrophilic, high molecular weight (MW), polyanionic compounds and ***do not cross the blood-brain barrier (BBB)***. Targeting antisense agents to brain cells is a “two barrier” targeting problem, because the antisense OGN must be targeted both through the BBB and the brain cell membrane, the target mRNA being localised within the cytoplasm [6,9,10].

Even *intracerebroventricular infusion (i.c.v.) of phosphorothioates (PS-ODN)* confers *very limited penetration into brain parenchima* (<100 $\square$m from the ependymal surface) and results in *significant neurotoxicity* caused by the strong reactivity of S atoms with multiple cellular proteins.

Therefore, neuropharmaceutical antisense OGN delivery to the brain might be achieved by **chimeric peptide technology** supposing their conjugation to brain-targeting vectors, which are transported from blood into the brain by absorbtive- or receptor-mediated transcytosis.

In chimeric peptide technology *the monobiotinylated antisense OGN is linked to a fusion protein* by means of the avidin-biotin strategy. *The fusion protein consists of either the BBB-targeting vector/avidin (AV) or BBB-targeting vector/streptavidin (SA)* [5,6,8].

The BBB-targeting vector might be: monoclonal antibody, highly cationized proteins, endogenous peptides or plasma proteins. An example of monoclonal antibody (MAb) specific to the receptors on BBB or on nervous cell membrane is MAb anti-transferrin receptor, codified OX26, responsible for receptor-mediated transcytosis. Cationized human serum albumin (cHSA) binds to the anionic sites of the brain capillary endothelium and triggers the

absorptive-mediated endocytosis and transcytosis through the BBB *in vivo*. Finally, endogenous peptides or plasma proteins are ligands for the receptor- or absorbtive-mediated systems at the BBB.

The avidin-biotin linker strategy [11,12], used in the chimeric peptide technology to couple the antisense oligonucleotide and the brain delivery vector, maintains the bifunctionality of the antisense/vector conjugate, that is, the biologic activity and the affinity for the BBB receptor. This linker strategy optimizes the plasma pharmacokinetic of the conjugate and improves its brain uptake as well. It also reduces the complex synthesis of a chimeric peptide to simple monobiotinylation of the antisense OGN, since vector/AV or vector/SA fusion protein may be largely-obtained by genetic engineering.

The avidin-biotin strategy requires monobiotinylation of the antisense OGN because multibiotinylation facilitates the formation of high molecular weight aggregates with AV or SA, responsible for the rapid removal from plasma and reduced brain uptake of the neuropharmaceutical antisense/BBB-targeting vector conjugates.

Thus, chimeric phosphodiesters (PO-ODN) and phosphorothioates (PS-ODN) are designed by the conjugation of 3'-biotinyl-OGN with a fusion protein, represented either by cationized human serum albumin/avidin (cHSA/AV) or by monoclonal antibody to rat transferrin receptor/streptavidin (OX26/SA). Chimeric PO-ODN and PS-ODN cross BBB, hybridize with complementary mRNA and activate RNase H (RNase H cleaves the target mRNA chain from its hybrid with the chimeric OGN), but are degraded by exonucleases *in vivo*.

Chimeric peptide nucleic acids

The significant problems associated with the use of chimeric PS-ODN, such as nonantisense interactions, serum proteins binding, *in vivo* metabolic instability and neurotoxicity, lead to the development of ***peptide nucleic acids (PNA) as neuropharmaceuticals*** [1, 4, 6].

PNA are electrically neutral, nontoxic, nuclease-resistant, not plasma protein-bound, have high affinity for target mRNA sequences, but are highly water-soluble molecules with MW of 5-10 kDa and too large to traverse the BBB by lipid-mediated transport (**Figure 1**).

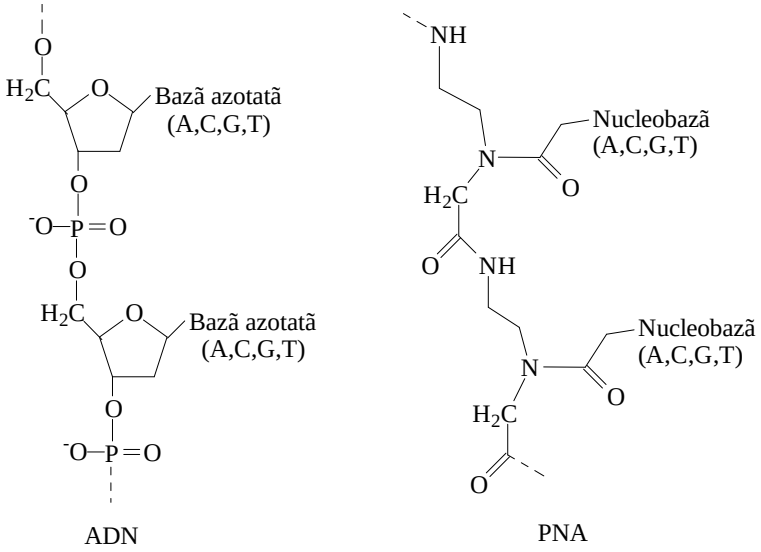

Figure 1. The structure of peptide nucleic acids (PNA)

The antisense activity and ability of the PNA chimeric peptide to hybridize to the target mRNA is unimpaired following conjugation of the biotinylated PNA to the anti-transfferin receptor monoclonal antibody OX26/SA vector as brain drug targeting system, using a noncleavable amide linker. The brain uptake of PNA chimeric peptide is approximately 3 log orders of magnitude greater than the brain uptake of the unconjugated PNA.

PNA chimeric peptides are the ***ideal antisense radiopharmaceuticals*** because they do not trigger cleavage of the target transcript.

Molecular formulation of PNA chimeric peptide [6,11,12] as antisense imaging agent comprises the following *domains*: (1) *the peptidomimetic monoclonal antibody* that targets an endogenous transport system on BBB and brain cell membrane or tumor cell membrane; (2) *streptavidin* as linker moiety which has high affinity for biotin incorporated at the amino-terminus of the PNA; (3) *the antisense sequence* of the PNA which hybridizes with the target mRNA; (4) *the radionuclide* attached to tyrosine-lysine residues at the carboxyl terminus of the PNA.

PNA chimeric peptide for imaging gene expression in the *human brain in vivo* uses as peptidomimetic MAb the *HIR MAb* (human insulin receptor monoclonal antibody) *or chimeric humanized HIR MAb,* because they are specific to humans, are nearly 10 times more active as BBB-drug targeting system than anti-transferrin receptor MAb, and HIR are also expressed on tumor cell plasma membrane (**Figure 2**).

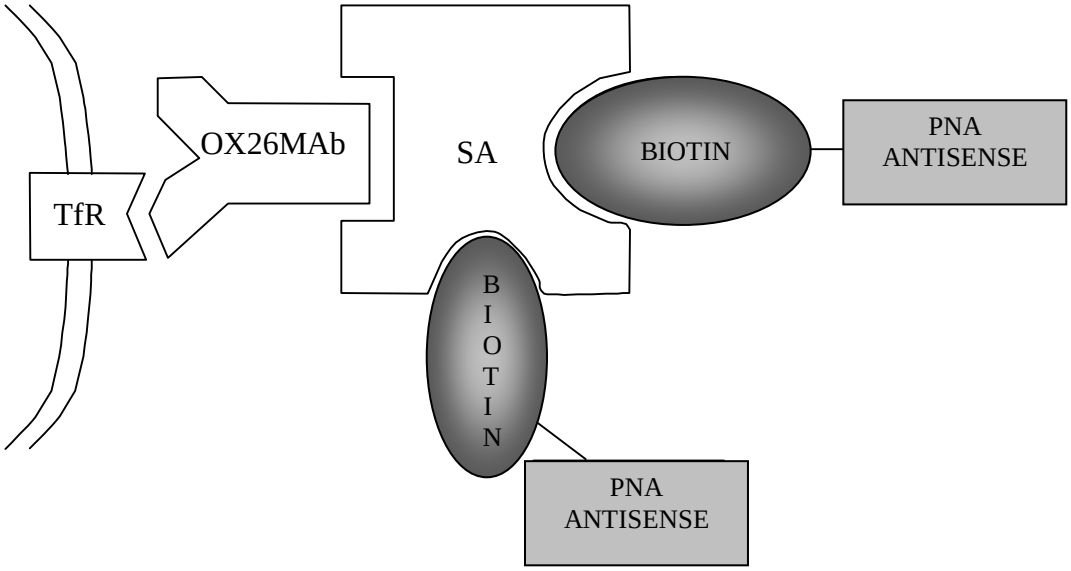

Figure 2. The structure of the PNA chimeric peptide as radiodiagnostic agent for brain tumors

It might be assumed that the development of *antisense radiopharmaceuticals* and their *adaptation to BBB drug-targeting strategy* represents the only way for *"direct imaging any pathologic gene in vivo in the brain of any person", moreover the early detection of the exact moment of its expression* [6].

Conclusions

Antisense neurotherapeutics might cure cancer, infectious or degenerative brain diseases and antisense radiopharmaceuticals are potential neurodiagnostic agents imaging the exact moment of any pathologic gene expression *in vivo*.

Antisense neuropharmaceuticals do not cross blood-brain-barrier and must be conjugated with brain-targeting vectors.

The chimeric peptide technology represents a brilliant strategy for improving the uptake of the antisense neuropharmaceuticals into targeted brain regions.

The development of the antisense neurotherapeutics and neurodiagnostics is paralled by brain drug targeting strategies.

References

1. CHIASSON BJ., HEBB MO., ROBERTSON HA., in *Antisense Research and Applications* (Crooke ST. Ed.), Springer-Verlag, 309-332, (1998).
2. ROGERS S.C., SULLENGER A.B., GEORGE L.A. JR., in Goodman & Gilman's *The Pharmacological Basis of Therapeutics*, (Hardman and Limbird Ed.), The McGraw-Hill Companies Inc., 10th edition, 81-112, (2001).
3. COHEN J.S., in *Biologic Therapy of Cancer* (DeVita V., Hellman S., Rosenberg S.A. Editors), J.B.Lippincott Company, 763-775, (1991).
4. CROOKE ST, BENNETT CF, *Annual Rev Pharmacol Toxicol*., **36**, 107, (1996).
5. QIN ZH., *Mol Pharmacol*, **48**, 730, (1995).
6. PARDRIDGE WILLIAM, *Brain drug targeting. The future of brain drug development,* Cambridge University Press, 221, (2001).
7. DAVIDKOVA G., WEISS B. in *Handb Exp Pharm*, **131**, 263, (1998).
8. MCMAHON BM., *Brain Res.*, **904**, 345, (2001).
9. BELTINGER C., *J.Clin. Invest*., **95**, 1814, (1995).
10. BROWN D.A., KANG S.H., GRYAZNOV S.M., *J. Biol. Chem*., **269**, 26801, (1994).
11. NOE R. C., KUAFHOLD L. in *New Trends in Synthetic Medicinal Chemistry* (Fulvio Gualtieri Ed.), Wiley-VCH Verlag GmbH, (2000).
12. ZHANG SP., *J Mol Neurosci*, **7**, 13, (1996).