

ANTISENSE OLIGONUCLEOTIDES, APTAMERS AND siRNA: PROMISES FOR THE TREATMENT OF OCULAR DISEASES

OLIGONUCLEÓTIDOS ANTISENTIDO, APTÁMEROS Y ARN-Si: PROMESAS PARA EL TRATAMIENTO DE ENFERMEDADES OCULARES

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The different strategies of inhibition of gene expression have been applied in many therapeutic applications among which the treatment of ocular diseases. Indeed, intraocular delivery of nucleic acid such as antisense oligonucleotides, aptamers or siRNA has raised a lot of interests in the recent years. Among the promising therapeutic approaches is antisense technology-oligonucleotides are designed to be complementary to a target RNA sequence so they can bind to the target and stop the production of undesirable proteins. This gene selectivity enables targeted drug design and therefore the production of more effective and less toxic therapeutics. The world's first and only antisense drug approved in November 1998 by the FDA has been Vitravene[®], an oligonucleotide discovered by ISIS for the treatment of cytomegalovirus retinitis in persons with AIDS. Vitravene[®] was marketed by Novartis Ophthalmics and came on the market just as powerful AIDS medications. Although, it changed the course of the disease, patients today rarely develop CMV, and Vitravene's sales have been extremely small. In addition, Vitravene[®] was shown to induce ocular inflammation as one of the major side effect. Despite, the lack of success of Vitravene[®], the company ISIS produced one other antisense oligonucleotides which was licensed to iCo Therapeutics. It is an antisense inhibitor of c-Raf kinase, an enzyme important in the signal transduction pathway triggered by the vascular endothelial growth factor (VEGF) and other important growth factors. In preclinical studies, antisense inhibition

of c-Raf kinase was associated with a reduction in the formation of new blood vessels in the eye, suggesting that c-Raf kinase inhibition could be valuable in the treatment of both age-related macular degeneration and diabetic retinopathy (1).

Other types of molecules that inhibit gene expression are the aptamers. These molecules are DNA or RNA molecules that have been selected from random pools based on their ability to bind other molecules. Aptamers can bind nucleic acids, proteins, small organic compounds, and even entire organisms. These novel molecules have shown many potential in the treatment of ocular diseases. The FDA has recently approved Macugen[®] (pegaptanib sodium injection) discovered by Eyetech Pharmaceuticals, Inc. Macugen[®] is designed to treat neovascular, or «wet», age-related macular degeneration (Partner Pfizer Inc. will market the drug) The new drug is a pegylated anti-VEGF aptamer, a single stranded nucleic acid that binds to the VEGF 165 protein, the specific type of VEGF that signals the growth of the abnormal new blood vessels. In the wet form of the disease, those new vessels leak blood and cause vision loss or even blindness. Macugen[®] acts as an antagonist and blocks the binding of VEGF to its receptor (2).

A more recent approach for targeting mRNA is the use of small interfering RNA or si RNA. siRNA are double stranded nucleic acids which are made of 21-23 nucleotides and able, intracellularly, to assemble to a multiprotein complex, termed RNA induced silencing complex (RISC). The RISC con-

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tains 1) a helicase activity that unwinds the two strands of RNA molecules, allowing the antisense strand to bind to the targeted RNA molecule and 2) an endonuclease activity which hydrolyzes the target mRNA homologous at the site where the antisense strand is bound. The small double-stranded RNAs (siRNAs) have appeared to be very efficient agents to inhibit gene expression in mammalian cells. Sirna-027 is a chemically modified short siRNA targeting VEGF Receptor-1 (VEGFR-1). VEGFR-1 is a key component of the clinically validated vascular endothelial growth factor (VEGF) pathway. VEGFR-1 is found primarily on vascular endothelial cells and is stimulated by both VEGF and placental growth factor (PIGF), resulting in the growth of new blood vessels. By targeting VEGFR-1, Sirna-027 is designed to shut down activation of pathologic angiogenesis initiated by both VEGF and PIGF. Sirna-027 was shown to inhibit neovascularization (new blood vessel growth associated with disease) in several validated preclinical models. Notably, these studies showed important effects at the molecular level as well, resulting in reduced levels of VEGFR-1 mRNA and protein (3).

As mentioned earlier, the target site of nucleic acids is in most cases the posterior segment of the eye. Free nucleic acids do not penetrate the cornea and remain confined to the superficial epithelial layer. Passive diffusion of drugs across the cornea is largely influenced by their solubility, molecular weight and degree of ionization. Nucleic acids are characterized by a high molecular weight and a negative charge being not able to pass across the cornea. To improve the transport through ocular tissues iontophoresis can be applied. Indeed, transcorneal iontophoresis system is a repeatable non-invasive method of delivery which facilitates markedly the intraocular penetration of oligonucleotides both in the anterior and posterior segments of the

eye. The application can be repeated as many times as necessary to achieve the needed continuous therapeutic levels without any danger or unwarranted side effects. As an alternative to transcorneal transport, delivery of nucleic acids to the intraocular tissues can be achieved using intravitreal administration. Intravitreal injection is a very delicate administration route. Indeed, the patient must remain under local anesthesia during injection since the needle must penetrate the vitreous humor without causing retinal disruption or detachment. Due to their poor stability in biological fluids, nucleic acids have a short intravitreal half-life. Their therapeutic application therefore requires repeated intraocular administrations to achieve a continuous intraocular presence of intact ODNs. Repeated intravitreal injections increase the risk of endophthalmitis, damage to lens, retinal detachment, and may be poorly tolerated. For this purpose, the use of long term delivery systems can be applied such as liposomes, implants or microspheres made of biodegradable poly(lactide-co-glycolide) polymers appears necessary to overcome these limitations. This last system was applied to antiVEGF aptamers allowing the release of about 2 µg/day over a period of 20 days with retained activity or to an antiTGFβ2 oligonucleotide to improve bleb survival in a rabbit experimental model of filtering surgery.

REFERENCES

1. Bochet A, Couvreur P, Fattal E. Intravitreal administration of antisense oligonucleotides: potential of liposomal delivery. *Prog Retin Eye Res* 2000; 19: 131-147.
2. van Wijngaarden P, Coster DJ, Williams KA. Inhibitors of ocular neovascularization: promises and potential problems. *JAMA* 2005; 293:1509-1513.
3. Gomes Dos Santos AL, Bochet A, Fattal E. Intraocular delivery of oligonucleotides. *Curr Pharm Biotechnol* 2005; 6: 7-15.