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Success for oral antisense therapy

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An oral antisense drug, designated EN101, that targets a variant form of acetylcholinesterase (AChE), has been shown to alleviate the symptoms of Myasthenia gravis in a Phase 1b clinical study [1].

Although the concept of oral antisense oligonucleotide therapies has held widespread appeal, it has also met with scepticism. Now, it appears that the long awaited proof-of-concept has arrived. Ester Neurosciences of Herzlia Pituach, Israel (http://www.esterneuro.com) recently reported the success of EN101 – the first oral antisense therapy. Neurologist and co-investigator Jon Sussman, at the Greater Manchester Neuroscience Centre of Hope Hospital in Salford (http://www.hop.man.ac.uk) regards the study as a 'beautiful model of how antisense might be used, as well as a potential new treatment for Myasthenia gravis'.

Muscle AChEs

Myasthenia gravis (MG) is a disease characterized by muscle weakness and fatigue resulting from faulty transmission at the neuromuscular junction. MG can result from a congenital abnormality but is more commonly an acquired autoimmune response. Normally, nerve endings release the neurotransmitter acetylcholine (ACh), which diffuses across the synapse and binds to ACh receptors on muscle cells, initiating muscle contraction. In the majority of patients with MG, antibodies to the ACh receptor cause loss or dysfunction of the receptor, thereby preventing ACh activity and resulting in apparent muscular weakness.

Symptoms of MG usually include droopy eyelids and double vision and can progress to weakness of the muscles of the limbs, as well as difficulty with speech, chewing, swallowing and breathing. The standard treatment includes AChE inhibitors, such as Mestinon®, which retard the normally rapid hydrolysis of ACh so that its concentration is increased in the synapse, thus overcoming the lack of functional receptors. ACh is expressed in other cell types and tissues; therefore, patients taking Mestinon® often experience side effects, such as diarrhea, stomach cramps and muscular cramps.

Making perfect antisense

An alternative strategy to traditional AChE inhibitors is to block AChE activity with an antisense oligonucleotide. The principle is simple: short reverse complementary sequences modify gene expression by hybridizing with target mRNA, forming a stable duplex that prevents translation and renders the mRNA susceptible to destruction by RNAse H [2]. 'In theory, antisense technology always looked promising for the treatment of various disorders but antisense oligonucleotide-based therapy faced many challenges, including poor stability in the body and the lack of specificity of action,' says Vinod Labhasetwar, Associate Professor of Pharmaceutics at the University of Nebraska Medical Center (http://www.unmc.edu), who is researching the oral delivery of oligonucleotides using biodegradable nanoparticles.

EN101 is a 20-mer oligonucleotide that is chemically modified by the insertion of 2′-oxymethyl groups at the three terminal positions of the 3′ end, thus preventing non-specific interactions with other targets and enabling EN101 to be effective at low doses. Sussman notes, 'The stability of the drug is so high that, although there are likely to be a comparatively small number of antisense molecules within any one cell, those molecules get recycled. They bind to the mRNA, the mRNA gets degraded and the antisense falls off and binds to the next molecule.'

Oral history

In this trial of patients with MG, Mestinon® therapy was discontinued and replaced by a once daily oral dose of EN101 for four days. 15 out of 16 patients demonstrated clear symptomatic improvement, as determined by the quantitative MG score, a standardized muscle strength-testing program. The improvements lasted for up to 72 hours following the final dose, a sharp contrast to the short-acting Mestinon® that must be taken up to five times per day to quell symptoms.

The results were pleasantly surprising because oral administration of antisense therapy has long been sought after but never achieved. Eli Hazum, CEO of Ester Neurosciences, admits, 'Frankly, it was very difficult for me to even imagine that we'd be able to give the antisense orally. As a matter of fact, we had designed our clinical studies to be in two arms, intravenous and oral; for some reason, we started with the oral administration and it worked.'

'Currently, there's only one licensed antisense drug and that's used to treat cytomegalovirus (CMV) infection of the retina. This drug has to be injected into the eyeball – so you can imagine the excitement when we give somebody something to drink and it sorts their problem out,' Sussman quips.

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Positive feedback

EN101 is also the first antisense agent targeted against an alternative splicing variant of a human gene. 'Numerous human syndromes (including aging-related diseases such as Alzheimer's) involve alternative splicing responses,' says Hermona Soreq, Head of the Eric Roland Center for Neurodegenerative Diseases at the Hebrew University of Jerusalem (http://www.huji.ac.il), 'therefore, antisense targeting to disease-associated splice variants is of special interest, especially as it offers sequence specificity that is unique to this technology,' [3,4]. EN101 targets the 'readthrough' AChE isoform, which is specifically upregulated in response to stress and following exposure to AChE inhibitors. Intervening at the pre-expression level with EN101 is thought to circumvent this feedback loop (Fig.1).

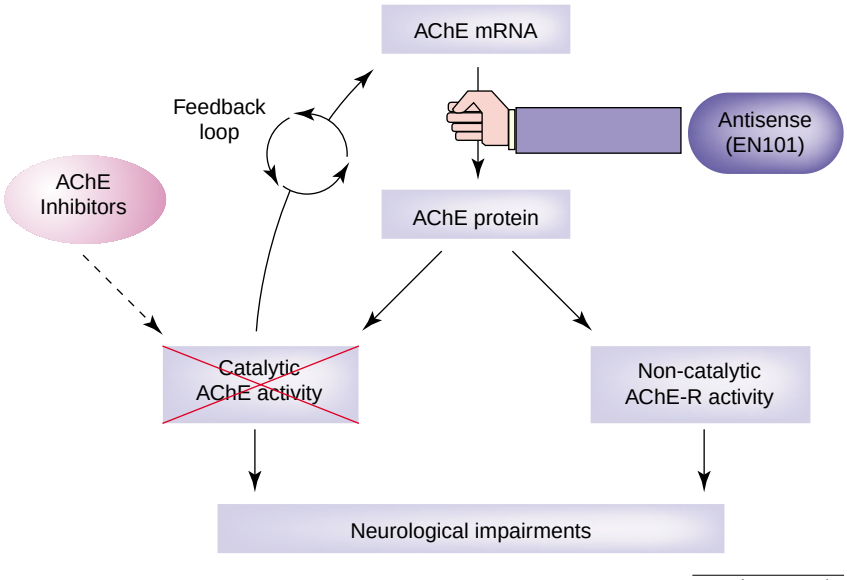

Figure 1. The advantage of pre-expression blockade with antisense. Conventional AChE inhibitors act on the catalytic properties of the synaptic isoform of acetylcholinesterase (AChE-S) but, according to Ester Neurosciences' findings, are themselves stressors, which cause the upregulation of the 'readthrough' isoform (AChE-R). Intervening at the pre-expression level with antisense oligonucleotide EN101 blocks the production of new AChE-R and prevents this destructive feedback loop, offering a higher probability of a disease-modifying level of efficacy. Figure courtesy of Eli Hazum, Ester Neurosciences (http://www.esterneuro.com).

Proof of concept

James Howard, Jr, Professor of Neurology and Medicine at the University of North Carolina in Chapel Hill (http://www.unc.edu), finds the results of the EN101 trial 'gratifying and exciting' but remarks that 'it would have been nice to see a parallel arm using the standard cholinesterase inhibitor.' Ester is planning to extend the trial and to develop EN101 for other peripheral nervous system disorders in which AChE has an important role, such as muscular dystrophy and multiple sclerosis.

Alan Gewirtz, Professor of Internal Medicine at the University of Pennsylvania School of Medicine (http://www.upenn.edu) uses antisense technology to examine the role of proto-oncogenes in human haematopoietic cell development. He aptly describes the potential for other antisense drugs, 'There's a whole world of people that want to use [antisense therapy] to treat cancer or cardiovascular disease…or diseases that involve any kind of cell growth or cell proliferation because, in theory, if you can regulate or silence the expression of genes that are driving the development of the cancer or driving an inflammatory process, then there's an endless number of applications.'

References

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