Less is more: therapeutic exon skipping for Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a lethal X-linked progressive muscle-wasting disease caused by premature truncation of the translation of DMD mRNA into dystrophin. Owing to improved respiratory support and treatment with steroids, patients can now survive into early adulthood, which is one or two decades longer than they could survive without such interventions. Despite much research worldwide, no disease-modifying treatment is yet available; however, promising results are being achieved with antisense oligonucleotides. Antisense oligonucleotides are modified DNA or RNA analogues that hybridise with a target DNA or RNA sequence, and they can be designed to prevent specific exons of DMD from being spliced into the mRNA. This so-called exon skipping can reframe the disrupted open reading frame of DMD to produce a shorter but functional protein (figure), such as the protein produced in individuals with the milder form of the disease, Becker muscular dystrophy. This less is more approach has been developed over the past decade in patient-derived cell cultures and in the mdx mouse model.

Modifications to the chemistry of antisense oligonucleotides are necessary to increase their half life and prevent the cleavage of RNA–RNA hybrids by RNase H, which is the goal of antisense-mediated knockdown of gene function but is unwanted for antisense-mediated modulation of splicing. The most commonly used chemicals for exon-skipping antisense oligonucleotides are 2′OMePS RNA with a negatively charged phosphorothioate backbone (2′OMePS) and uncharged phosphorodiamidate morpholino oligomers (PMO), because both are stable and non-toxic.

In 2007, an intramuscular proof-of-concept clinical study was reported by van Deutekom and co-authors. They used PRO051, a 2′OMePS RNA that targets exon 51. Skipping of exon 51 corrects several mutations in the DMD deletion “hotspot”, which amounts to about 13% of the mutations that cause DMD. In the September issue of The Lancet Neurology, Kinali and co-authors report the results of a similar proof-of-concept study with intramuscular AVI-4658, a PMO that also targets exon 51. Although there are similarities and differences between the two studies, the main combined messages are that there are no drug-related adverse effects and that both backbone chemistries have about equal effectiveness. These findings are valuable because they imply that there is more freedom in the choice of chemistry than has been known so far. However, although nucleic acid-based drugs, such as PMO or 2′OMePS, are promising because of their apparent low toxicity, and they have not yet been tested over the prolonged period of administration required for treating childhood disease.

The table compares two studies. van Deutekom and co-authors injected a 2×1 cm area of the tibialis anterior muscles, and in the September issue of The Lancet Neurology, Kinali and co-authors injected a 3×1 cm area of the tibialis anterior muscles. The main differences between the two studies are that van Deutekom and co-authors administered the oligonucleotide once, whereas Kinali and co-authors administered it twice.

Figure 1: Antisense-mediated exon skipping to reframe DMD transcripts

Patients with Duchenne muscular dystrophy have mutations in DMD, the gene that encodes dystrophin. The mutations disrupt the open reading frame of dystrophin (in this example, exons 45–50 are deleted). Consequently, protein translation stops prematurely, resulting in a non-functional protein. By use of antisense oligonucleotides that target a specific exon in which there is a mutation that truncates the expression of dystrophin (exon 51 in this example), the reading frame can be restored. This enables the production of an internally deleted but partially functional dystrophin. AON—antisense oligonucleotide.
muscle in the lower leg, but took only a small biopsy of the muscle 4 weeks after treatment. Kiniali and co-authors targeted a 1 cm² area of the extensor digitorum brevis, a small muscle in the foot, and the muscle was completely removed for analysis; they also biopsied the saline-injected contralateral muscle. Kiniali and co-authors showed that low-dose AVI-4658 did not induce expression of dystrophin that was greater than the background expression, whereas high-dose AVI-4658 induced a significant increase in dystrophin expression and the number of dystrophin-positive fibres seen with immunohistochemical staining.

The proportion of dystrophin-positive fibres and the amount of dystrophin detected vary between the studies but, owing to the differences in several parameters (eg, subtraction of expression in contralateral control muscle, the volume of muscle that is injected [and hence, the local expression]), and the selection for analysis of fascicles that are adjacent to the injection track), not much significance should be attributed to these differences in results. Kiniali and co-authors summarise it best when they say “both studies reported unequivocable expression of dystrophin at similar concentrations”; moreover, both are proof-of-concept studies. The next step, which both groups are currently undertaking, is to deliver the antisense oligonucleotide systemically; therefore, detailed comparisons of intramuscular results are largely irrelevant. Only systemic trials will reveal the true promise of this approach, and further trials are needed to validate the functional benefit, or at least the decline in disease progression. Indeed, any resolution as to which chemistry (if either) is the best choice should wait until after systemic comparisons are made, under conditions that are otherwise identical. Notably, although delivery is the main obstacle to the clinical application of many antisense-based treatments, the pathophysiology of DMD benefits delivery, and uptake of antisense oligonucleotides is much better in damaged, dystrophic muscles than it is in healthy muscle.

Finally, the sequence-specific approach has implications for future personalised therapy. Although skipping of exon 51 is applicable for 13% of patients with Duchenne muscular dystrophy, it will not benefit the other 87%, whereas skipping of 10 exons might be beneficial for more than 70% of patients with a deletion mutation in DMD, or 40% of all patients. Other approaches are being developed that could raise the number of patients who can be treated: for example, antisense oligonucleotides to duplications and point mutations through double-exon11,12 and multi-exon skipping.13,14 Although these approaches look promising on paper, not all are successful in practice, and we might need to learn much more about the complex splicing of this large gene (2·5 Mbp and 79 exons) to optimise its therapeutic correction. In all cases, this process will require close coordination with regulatory agencies because there are too few patients with the rarer mutations to set up independent clinical trials.
Cervicogenic headache: a pain in the neck for some neurologists?

In this issue of The Lancet Neurology, Bogduk and Govind review the vexed topic of cervicogenic headache. As a neurologist interested in headache, it seems self-evident that this topic should be of interest. Much of what has passed for science in this field is rightly criticised by the authors, and there seems no benefit in trawling over the arguments, for if there was no controversy there would be no issue. As the authors say, the anatomy and physiology are clear enough, so what is the problem? There are several issues, again largely and capably discussed by Bogduk and Govind; I would like to highlight some of these from the neurologist’s perspective, as I think we need to get our house in order. The three particular topics of note are: the clinical implications of the anatomy and physiology, the problem of referral bias, and the issue of primary versus secondary headache from a neurologist’s point of view. Finally, how should we proceed?

Perhaps the most basic issue revolves around the anatomy and physiology of upper cervical segment nociceptive afferents and their projections to second-order neurons. In experimental animals, the cervical and ophthalmic division of trigeminal neurons clearly synapse on common second-order neurons in the trigemino-cervical complex. The basic data are supported by clinical observations, such as referred pain from cervical muscles and the C2–3 zygapophysial joint. Interestingly, the clinical data reviewed suggest that the caudal limit of cranial referral in the neck is at the level of the C3 afferents; this is certainly consistent with the laboratory anatomy. What would the convergence of these afferents predict? The data suggest that nociceptive activation in structures innervated by either trigeminal or upper cervical afferents might result in a perception of pain that is not anatomically related to the structure with pathology. Put simply, the anatomy predicts that the simple clinical localisation process of “pain marks the spot” is doomed to fail. Moreover, the data all suggest that cervicogenic headache—headache from activation in structures innervated by either trigeminal or upper cervical afferents—will be swamped with cases of migraine. If the above assumption does not mean cervicogenic headache does not exist, it does not demean it, nor imply anything negative; this is just referral bias. In a recent retrospective study of patients who responded to occipital nerve...