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ALS, IGF-1 and gene therapy: 'it's never too late to mend'

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease characterized by the selective and progressive loss of motor neurons in the spinal cord, brainstem and cerebral cortex. It typically leads to progressive muscle weakness and neuromuscular respiratory failure. Approximately 20% of the familial forms of ALS are associated with point mutations in the gene coding for the Cu/Zn superoxide dismutase-1 enzyme (SOD1). The discovery of this primary genetic cause of ALS has provided a basis for testing various therapeutic possibilities.

The potent neuroprotective activities of neurotrophic factors (NTFs), ranging from prevention of neuronal atrophy, axonal degeneration and cell death, generated a great deal of hope for the treatment of ALS in the early 90s. Ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1) have already been evaluated in ALS patients. The rationale for testing these factors in ALS patients was based on their trophic effects on naturally occurring cell death paradigms during development, traumatic nerve injury or in animal models resembling ALS such as *pmn* or *wobbler* mice. Disappointingly, systemic delivery of these recombinant proteins did not lead to clinically beneficial effects in ALS patients.[1] Undesirable side effects and limited bioavailability have complicated the evaluation of their potential clinical benefits.

Contemporaneously, several strains of transgenic mice overexpressing different ALS-linked SOD1 mutations have been generated.[2] By closely mimicking many of the clinical and neuropathological features of ALS, these mice have provided more relevant animal models for investigating the preclinical potential of neurotrophic factors. Direct administration of recombinant trophic proteins has been disappointing. Beneficial effects on motor neuron neuropathology are subtle or null.[3–5] Viral vector-mediated delivery of neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) or cardiotrophin-1 (CT-1) also did not lead to clear behavioral or neuropathological improvement.[6,7] Due to these previous disappointing results, the publication of Kaspar *et al*[8] in a recent *Science* issue has generated a great deal of excitement. These authors report that a viral vector-mediated delivery of IGF-1 leads to an impressive beneficial effect on the natural history of the disease. Nonpathogenic adeno-associated viruses (AAV) carrying genetic instructions for IGF-1 expression were injected into the hindlimb quadriceps and intercostal muscles of transgenic mice expressing the G93A mutant SOD1. When administered at presymptomatic stages, IGF-1 showed its most remarkable effect on disease onset and survival. Perhaps more relevant from a clinical point of view, IGF-1 retained its protective action, although less robust, when $SOD1^{G93A}$ mice were injected with AAV-IGF-1 at disease onset. Taken together, these results not only celebrate the comeback of an 'old friend', IGF-1, but also rejuvenate the interest of gene therapy for neurodegenerative diseases.

A key question is to understand the reason for the success of this gene therapy approach. What is so special about delivering IGF-1 through AAV muscle infection? NTFs expressed through viral vector-mediated muscle infection may gain access to target cells via three possible routes: (1) systemic delivery, (2) retrograde transport of viral particles and (3) retrograde transport of biologically synthesized NTFs themselves. Surprisingly, the level of circulating IGF-1 measured in the plasma was not significantly higher in AAV-IGF-1 compared to non-treated mice. Previously published experiments based on AAV muscle infection suggest that this should have been the case.[7,9] Kaspar *et al* report that the viral vectors gain access to the spinal compartment and motor neuron soma through a retrograde transport along the afferent motor axons. Despite a very low percentage of viral particles retrogradely transported (approximately 1%), it appears that even scattered, transduced motor neurons, and also possibly sensory neurons, acted as neurotrophic micropumps spreading the trophic molecules to surrounding cells. When IGF-1 is produced solely by muscles (demonstrated by the use of a lentiviral vector that cannot be retrogradely transported), the neuroprotective effect is reduced. This observation suggests that IGF-1 does not act only as a peripheral factor, but also must be secreted directly in the spinal cord. It is conceivable that cells other than motor neurons may be influenced by IGF-1. The recent report that non-neuronal cells intervene in the disease process in fact supports this hypothesis.[10] A careful analysis of the effect induced by the expression of IGF-1 limited to the spinal cord level should be performed to corroborate this intriguing observation. These experiments can be achieved by direct intraspinal administration[11] or by intramuscular injection of viral vector driving the expression of IGF-1 under a neuron-specific promoter. A strong expression of IGF-1 can be achieved with a muscle-targeted viral vector; the IGF-1 can then be taken up at neuromuscular junctions and transported back to motor neurons through a retrograde transport mechanism. This retrograde delivery must have also accounted for disease progression as a muscle-restricted expression of IGF-1 also showed a positive impact. In the light of these experimental results, it seems that both muscular and spinal cord expression of IGF-1 act synergistically to promote motor neuron survival (Figure 1).

Previous clinical trials based on systemic delivery of a recombinant form of IGF-1 led to variable results.[12,13] Physiological hindrances and short half-life linked to circulating IGF-binding proteins might explain these disappointing results.

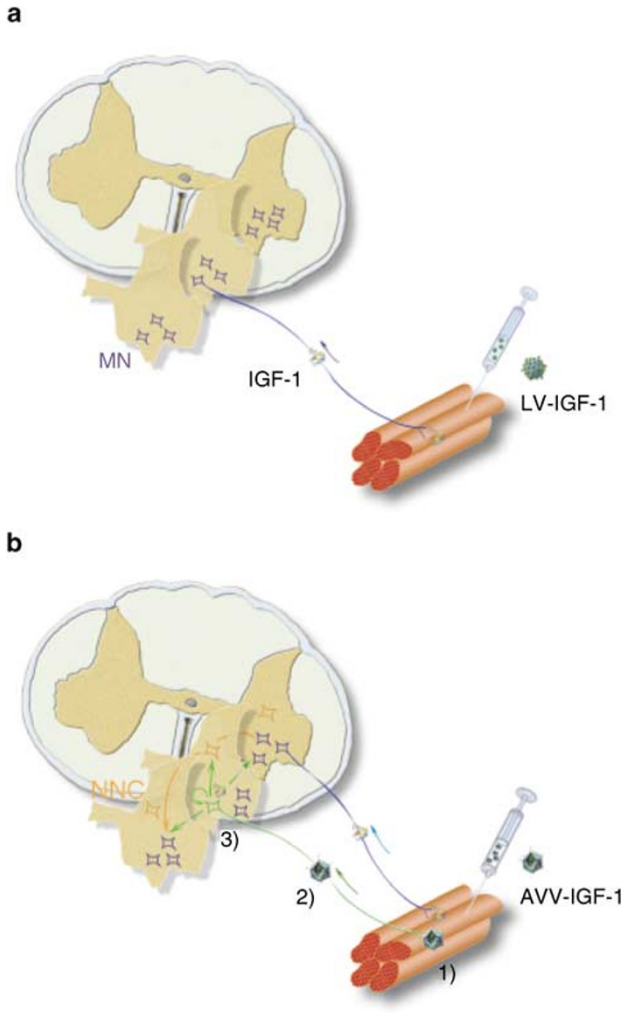

Figure 1 *Importance of IGF-1-secreting cellular platforms for providing neurotrophic benefits. (a) Intramuscular delivery of LV-IGF-1 leads to a muscle-restricted expression of IGF-1 that might be retrogradely transported to motor neurons (MN) and counteracts neuronal loss in ALS transgenic mice. In this case, the IGF-1 protective effect is less robust than in (b), where Adeno-associated virus can be taken up at nerve endings (1) and transported back to the spinal compartment to reach the motor neuron soma (2). AAV-IGF-1-transduced motor neurons act as micropumps that liberate IGF-1 in the spinal cord (3). IGF-1 trophic effect may involve autocrine and paracrine mechanisms. Non-neuronal cells (NNC) could also benefit from IGF-1 and give rise to protective signal(s) to the surrounding cells.*

IGF-1 delivery based on a gene therapy strategy appears to be a promising therapeutic approach. Its application in humans will, however, not be straightforward. Particularly challenging will be the issue of 'scale-up'. How many muscles will have to be injected? How many injections per muscle, not to say how many viral particles per injection site? Development of clinical trials will take advantage of recent advances in both the development of the optimization of viral vectors retrograde axonal transport by means of rabies-G pseudotyped lentivirus, and also vector development that allows tightly controlled transgene expression.[14–16] This expanding ensemble of gene therapy knowledge represents a vector of hope for future ALS clinical trials.

For further information about ALS and ALS research, http://www.alscenter.org and http://www.alsa.org. ■

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