Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Biotherapies in neurological diseases

Biotherapies of neuromuscular disorders

Biothérapies des maladies neuromusculaires

J.-F. Briand [*], *M.-O. Roy*, *F. Mourlane*, *C. André*, *N. Loux*, *C. Rougeau*, *T. Toursel*, *S. Braun*

AFM-Telethon, 1, rue de l'Internationale, BP 59, 91002 Evry cedex, France

INFO ARTICLE

Article history:
Received 14 April 2014
Accepted 25 July 2014
Available online 13 November 2014

Keywords:
Biotherapies
Gene therapy
RNA modulation
Muscle
Motoneuron
Monoclonal antibodies

Mots clés :
Biothérapies
Thérapie génique
Modulation de l'ARN
Muscle
Motoneurone
Anticorps monoclonaux

ABSTRACT

This review focuses on the most recent data on biotherapeutic approaches, using DNA, RNA, recombinant proteins, or cells as therapeutic tools or targets for the treatment of neuromuscular diseases. Many of these novel technologies have now reached the clinical stage and have or are about to move to the market. Others, like genome editing are still in an early stage but hold great promise.

RÉSUMÉ

Cette revue est consacrée aux données les plus récentes sur les approches thérapeutiques basées sur l'ADN, l'ARN, les protéines recombinantes ou les cellules en tant que cible ou agent médicamenteux pour le traitement des maladies neuromusculaires. Beaucoup de ces technologies novatrices ont atteint le stade clinique et ont ou sont sur le point d'atteindre la mise sur le marché. D'autres telles que l'ingénierie du génome sont encore à un stade précoce mais se révèlent très prometteuses.

1. Introduction

Neuromuscular disorders represent an heterogeneous group of around 300 inherited or acquired clinical disorders that primarily affect one or more components of the motor unit (motoneuron and the group of skeletal muscle fibers each motoneuron activates) [1]. Their treatment must overcome several challenges:

- the need to correct and preserve, in a durable manner, the majority of the target tissue (mainly the muscle) which

* *Corresponding author.*
E-mail address: jfbriand@afm-telethon.fr (J.-F. Briand).
http://dx.doi.org/10.1016/j.neurol.2014.07.018

represents half of the total mass of the organism, and the peripheral nervous system with specific bioavailability issues;
- many serious disorders beginning in infancy and/or evolving towards a progressive disappearance of functional tissue;
- a large variety of genetic anomalies which may be unique to each patient [2].

Nevertheless, recent progress in our understanding of the pathophysiology of neuromuscular disorders is now resulting in a number of new therapeutic concepts, including gene and/or cell-based products that offer new hopes for those mainly unmet medical needs.

Genetic engineering has made possible the targeted, specific modification of the genetic information of living organisms, either by transferring and expressing exogenous genetic or cell material in vivo, as well as to pharmacologically interfere with the functioning of the genome. This involves the correction or modulation of genes, both at the DNA and the RNA levels, and the delivery of regenerating cells, locally or systemically.

The present review updates the different strategies and achievements in the field.

2. Gene therapy of skeletal muscles

The first completed gene therapy clinical trial in the neuromuscular field was also the simplest conceptually. It used the local injection of a plasmid vector in Duchenne muscular dystrophy (DMD) patients. DMD is a fatal disease caused by mutations in the *dystrophin* gene leading to progressive muscle weakness and wasting. The non-viral, full-length *dystrophin* plasmid injected at very low dose (600 μg), showed low and local (along the needle track) expression of dystrophin, with no immune response to either the DNA or the transgene product [3]. Massive intravascular delivery in isolated limbs referred to as hydrodynamic limb vein (HLV) injection of the plasmid showed a much higher transfection efficiency (up to 40% of transfected muscles) in primate, and in mouse (*mdx*) and dog (golden retriever muscular dystrophy [GRMD]) DMD models [4]. Higher efficiency was obtained using adeno-associated viruses (AAV) [5]. As most viruses cannot accommodate sequences larger than 6 kb, mini-versions of the dystrophin gene are now being tested in the context of AAV to be delivered locally (phase I/II clinical trial ongoing in DMD patients [6]), systemically, or through HLV [2,7]. Safety of the locoregional HLV delivery of increasing volumes of buffer is in progress in a series (limb-girdle muscular dystrophy 2A, Emery-Dreifuss muscular dystrophy, autosomal recessive LGMD, autosomal dominant LGMD, and Becker muscular dystrophy) of volunteers [8]. They echo safety trials of local injections with AAV vectors in LGMD2D [9,10]. In order to circumvent the potential immune rejection of the therapeutic protein in naïve patients, gene therapy using unaffected homologs of the target protein are envisaged, such as utrophin, a close homolog of dystrophin [11].

Alternatively to mini-genes, human artificial chromosomes (HAC) vectors with the capacity to carry large genomic loci and to replicate and segregate autonomously without integration into the host genome, have been designed. HAC vectors containing the entire human *dystrophin* gene (DYS-HAC) with its native regulatory elements allow dystrophin expression at levels similar to native dystrophin isoforms expression levels. They could be introduced into patient stem or progenitor cells for ex vivo therapy [12].

Trans-splicing between distinct pre-messengers has also been proposed. In two similar approaches [13,14] three different AAV vectors were constructed with one third of the complete mouse dystrophin coding sequence flanked with inverted terminal repeat sequences. They allowed low levels of full-length dystrophin in the injected muscles of *mdx* or *mdx4cv* mice. Another trans-splicing approach is to replace mutated exons, to insert missing exons or to correct duplicated exons, with vectors carrying either sequences that, introduced in the mRNA, replace its mutated sequence, or antisense sequences specific of the intronic region upstream of the mutation [15]. Immunogenicity of the newly expressed protein or conformational epitopes of chimeric proteins cannot however be excluded.

Non-specific approaches based on myostatin inhibition to increase muscle mass are currently under investigation in patients with Becker muscular dystrophy or sporadic inclusion body myositis injected with an AAV1-CMV-human follistatin vector into the quadriceps muscle [16]. This muscle enhancement strategy could be used in combination with defective gene-targeted therapies.

Recently, spectacular survival outcome was achieved in X-linked myotubular myopathy (XLMTM), a rapidly fatal congenital pediatric disease that affects the entire skeletal musculature. Intravascular administration of a single dose of a recombinant AAV8 vector expressing murine or canine myotubularin into myotubularin-deficient mice and XLMTM dogs at the onset or at late stages of the disease resulted in robust improvement in motor activity and contractile force, corrected muscle pathology, and prolonged survival in the absence of toxicity or humoral or cell-mediated immune response. These results provide proof of concept for future clinical trials in muscle enzyme-deficient patients [17].

3. Gene therapy of motoneurons

Very few vector systems seem to be able to cross the blood-brain barrier. Among vectors, self-complementary AAV 9, and AAV10 showed good transduction efficiency following intravenous, intrathecal or intracisternal delivery, in mice, cats and monkeys, and they allowed transgene expression in glial cells throughout the brain, in dorsal root ganglia neurons and motor neurons within the spinal cord, as well as in skeletal muscle and peripheral organs [18–20]. This opens a new avenue (now at the phase I/II stage) for acquired or genetic diseases of the motoneuron, such amyotrophic lateral sclerosis (ALS) or spinal muscular atrophy (SMA), as well as lysosomal storage disorders affecting the central nervous system [9,21,22].