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Spinal muscular atrophies – distinctions and therapeutic progress

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Foreword

"Filling the Gaps in Drug Therapy" is a new section calling attention to diseases and conditions, some rare and others all too common, for which no effective therapy currently exists, or for which existing therapies are poorly tolerated or otherwise unsatisfactory. This month we present a brief overview of spinal muscular atrophies.

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Abstract

The motor neuron diseases spinal muscular atrophy (SMA) and spinobulbar muscular atrophy (SBMA) present with distinct etiologies and pathologies. SMA is an autosomal recessive genetic disease caused by mutation or deletion of the survival of the motor neuron (*SMN*) gene that transcribes the SMN protein, which is critical for the survival and health of motor neurons and brain stem nuclei. Therapeutic approaches to SMA involve replacement of SMN protein levels, which at present focus on the correction of *SMN2* gene splicing, upregulation of *SMN2* copy numbers or overall transcription and delivery of SMN protein to motor neurons via gene therapy. Stem cell therapy to promote motor neuron regeneration may also be beneficial. X-linked adult-onset SBMA is caused by a mutation in a region of the X chromosome that encodes an abnormal polyglutamine (polyQ) tract expansion in the androgen receptor (AR), making the mutated AR toxic to nerve cells. Current therapeutic targeting centers on reducing mutant AR-mediated neuronal toxicity by inhibiting nuclear translocation of the mutant AR and encouraging clearance of the 'toxic substance'. Distinguishing these neurodegenerative disorders is therefore important for successful therapeutic targeting and advances.

Introduction

Spinobulbar muscular atrophy (SBMA; also known as Kennedy's disease or Kennedy's syndrome) is often classified as a subtype of spinal muscular atrophy (SMA) despite their distinct etiology and pathology. Distinguishing these neurodegenerative disorders is, however, important for successful therapeutic targeting and advances. Treatment for both syndromes is, at present, usually symptomatic, although recent studies are filling in the gaps to provide targeted neuroprotective therapies. Table I presents therapeutic candidates for SMA and SBMA.

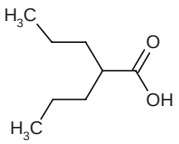
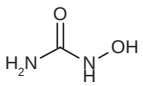
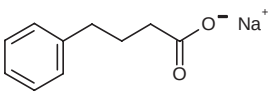
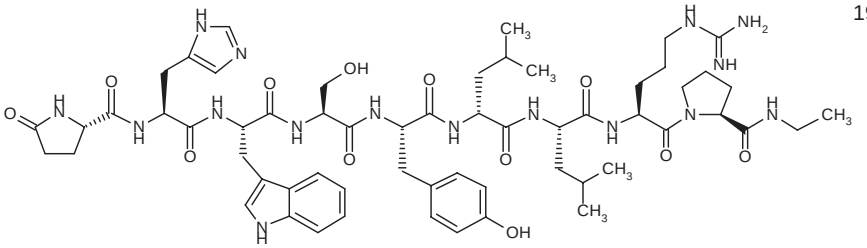
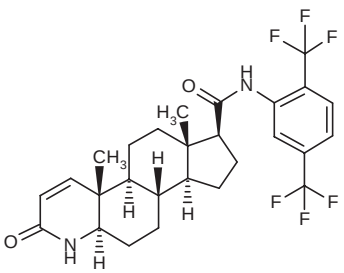
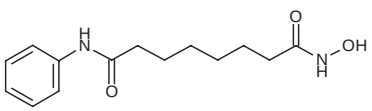
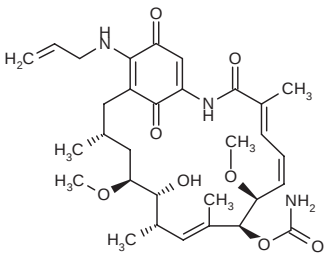
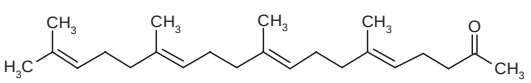
Spinal muscular atrophy

SMA is an autosomal recessive genetic disease that is characterized by progressive degeneration of motor neurons in the ventral (anterior) horn of the spinal cord, affecting voluntary muscles of the trunk and limbs to cause muscle weakness and difficulties in movement, breathing, speech, eating and swallowing, progressively leading to paralysis. It is a relatively common 'rare disorder' (the second most common fatal autosomal recessive disorder after cystic fibrosis) that affects 1 in 10,000 births, with approximately 1 in 50 people identified as genetic carriers (1). Several types of SMA exist, which are subdivided according to age or clinical severity. The most common are: type I (also known as Werdnig-Hoffmann disease; evident in the fetus or within the first few months following birth), type II (3-15 months), type III (also known as Kugelberg-Welander disease; 2-17 years of age) and type IV (adult-onset).

Targets and therapeutic advances

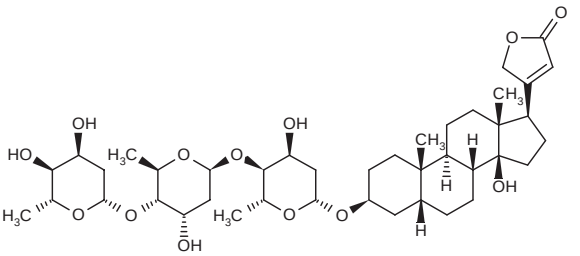
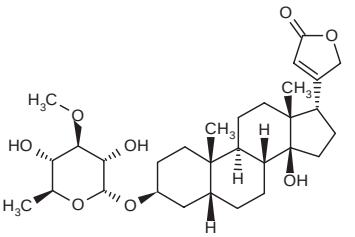
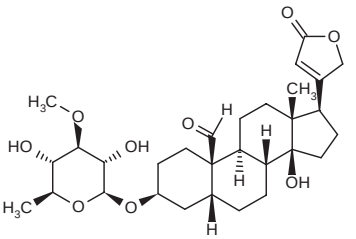
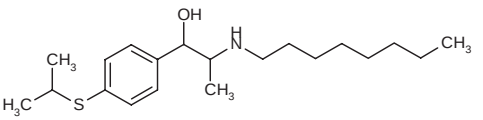
SMA is caused by deletion or mutation of the survival of motor neuron (*SMN*) gene that transcribes the SMN protein, which is critical for the survival and health of motor neurons and brain stem nuclei. In normal cases two *SMN* genes exist: *SMN1* and *SMN2*. The absence of or a defect in the *SMN1* gene causes SMA (2), as *SMN1* is the primary manufacturer of the full-length form of SMN protein. *SMN2* cannot compensate for dramatic reductions in SMN protein, as differential splicing of this gene predominantly produces a biologically inactive protein

Table I: Chemical structures of therapeutic candidates for SMA and SBMA (from Prous Science Integrity®).

	Drug	Structure	Ref.
SMA	Valproic acid		7, 9
	Hydroxyurea		8, 10
	Sodium phenylbutyrate		11
SBMA	Leuprolide acetate		19, 20
	Dutasteride		21
	Vorinostat		23
	17-AAG		24
	Teprenone		26

Continuation

Table I (cont.): Chemical structures of therapeutic candidates for SMA and SBMA (from Prous Science Integrity®).

	Drug	Structure	Ref.
SBMA	Digitoxin		28
	Nerifolin		28
	Peruvoside		28
	Suloctidil		28

isoform that lacks exon 7 (due to a C-to-T transition on exon 7) (3). However, SMA patients do seem to retain their *SMN2* allele and increased *SMN2* copy numbers have been shown to correlate with reduced SMA severity and improved duration of survival (4).

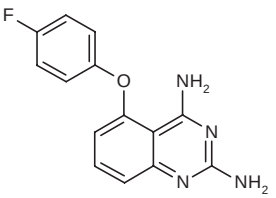
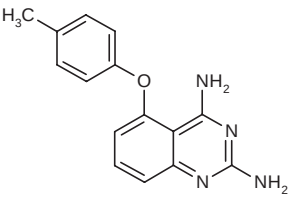
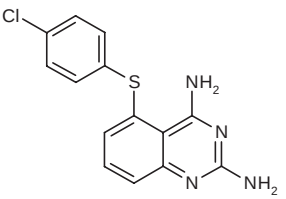
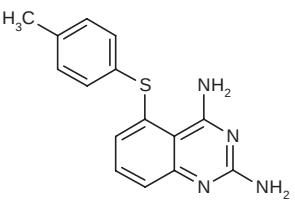
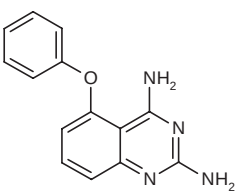
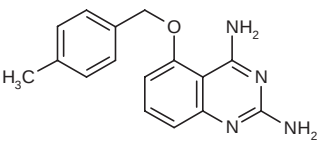
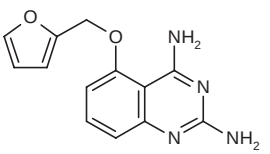
Some therapeutic approaches to SMA involve correction of *SMN2* gene splicing. Recent studies have demonstrated the replacement of SMN protein levels via the promotion of exon 7 inclusion in the *SMN2* gene using antisense technology. This method can achieve significantly and reproducibly elevated levels of functional SMN protein from the *SMN2* locus (5, 6), and may therefore be useful for modulation of the SMA phenotype.

As mentioned above, *SMN2* copy numbers can determine disease severity, and therefore another avenue of therapeutic investigation centers on upregulation of overall *SMN2* transcription. Histone deacetylases (HDAC) epigenetically regulate gene transcription by modifying histones. Correspondingly, HDAC inhibitors such as valproic acid (7) and hydroxyurea (8) have been shown to upregulate SMN protein by activating *SMN2* gene promoters. These HDAC inhibitors are currently under phase II (9) and II/III (10) clinical evaluation for SMA, respectively. Preliminary clinical efficacy for HDAC inhibitors in SMA has been demonstrated using sodium phenylbutyrate, an FDA-approved drug that upon oral administration elevates *SMN* gene expression in patient leukocytes (11).

In the patent literature, Vertex has described new compounds that could be useful as *SMN2* promoters (12), and deCODE Chemistry has claimed 2,4-diaminoquinazolines that increase the production of SMN2 without the undesirable side effects of valproic acid, such as liver toxicity (13). These compounds are displayed in Table II.

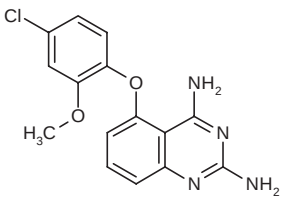
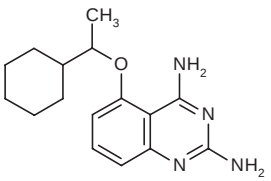
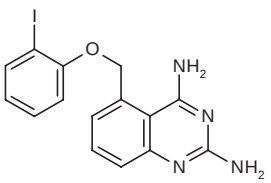
An alternative and attractive therapeutic option involves gene therapy to deliver SMN protein to motor neurons. A recent study demonstrated effective restoration of SMN protein levels in SMA type 1 fibroblasts via *in vivo* application of a lentivector gene transfer system, with associated reduced motor neuron death and prolonged animal survival (14).

Table II: Chemical structures of representative SMN2 promoters from patent literature (from Prous Science Integrity®).

Patent	Chemical name	Source	Structure	Ref.
WO 2004113305	5-(4-Fluorophenoxy)quinazoline-2,4-diamine	Vertex		12
	5-(4-Methylphenoxy)quinazoline-2,4-diamine			
	5-(4-Chlorophenylsulfanyl)quinazoline-2,4-diamine			
	5-(4-Methylphenylsulfanyl)quinazoline-2,4-diamine			
	5-Phenoxyquinazoline-2,4-diamine			
WO 2005123724	5-(4-Methylbenzyloxy)quinazoline-2,4-diamine	deCODE Chemistry		13
	5-(Furan-2-ylmethoxy)quinazoline-2,4-diamine			

Continuation

Table II (cont.) Chemical structures of representative SMN2 promoters from patent literature (from Prous Science Integrity®).

Patent	Chemical name	Source	Structure	Ref.
WO 2005123724	5-(4-Chloro-2-methoxyphenoxy)quinazoline-2,4-diamine	deCODE Chemistry		13
	5-(1-Cyclohexylethoxy)quinazoline-2,4-diamine			
	5-(2-Iodophenoxymethyl)quinazoline-2,4-diamine			

A non-SMN-targeting approach comprises stem cell therapy to restore neurons following degeneration. Recent studies in an animal model of SMA with respiratory distress type 1 (SMARD1) provided positive data following the intrathecal application of a spinal cord neural stem cell population isolated on the basis of aldehyde dehydrogenase (ALDH) activity. Stem cell transplantation delayed disease progression, spared motor neurons and ventral (anterior) root axons and increased lifespan (15).

Spinobulbar muscular atrophy

SBMA is an X-linked inherited adult-onset motor neuron disease that occurs in approximately 1 in 40,000 males (although it is thought to be severely under- and misdiagnosed). It is caused by a mutation in a region of the X chromosome encoding the androgen receptor (Xq11-12), and therefore affects responses to the androgen hormones such as testosterone. This process evokes progressive nerve cell death in the motor neurons of the bulbar region of the brain stem and the ventral (anterior) horn of the spinal cord (16), leading to muscle weakness and atrophy, with neurological symptoms typically occuring between 30 and 50 years of age. Weakness predominantly affects muscles in the proximal areas of the body, and is especially problematic in the muscles of the face and throat.

Targets and therapeutic advances

Physical and speech/swallowing therapy can be implemented to improve the symptoms of SBMA, although alternative pharmacological treatments are emerging as a result of investigation into the mechanisms underlying SBMA pathogenesis.

The X chromosome mutation associated with SBMA has been identified as a trinucleotide repeat expansion of CAG, which creates an abnormal polyglutamine (polyQ) tract expansion within transcribed androgen receptors (AR), making the mutated AR toxic to nerve cells. It is thought that the more CAG repeats are present, the more severe the disease. The mechanism by which this type of mutation causes neuromuscular disease is not completely understood, although extensive research has generated several theories that could be targeted therapeutically.

Most polyQ diseases display nuclear inclusions (NIs), which are considered to be relevant to the pathophysiology. In SBMA patients, NIs containing the mutant AR are detected in the motor neurons, yet various lines of research have suggested opposing hypotheses: 1) accumulation of mutant protein is essential for the induction of neuronal cell degeneration (17); and 2) NIs may represent a cellular defense mechanism to sequester the toxic proteins (18). However, as nuclear translocation of the mutant AR is ligand-dependent, further studies have indicated that testosterone accelerates nuclear translocation and neuronal toxicity, while leuprolide acetate (see Table I), which inhibits testosterone secretion, rescues the polyQ phenotype in a mouse model of SBMA (19). This suggests the therapeutic potential of hormonal intervention for SBMA, and leuprolide has recently completed phase II studies for improving muscle strength in SBMA (20). A phase II clinical study, run by the National Institute

of Neurological Disorders and Stroke (NINDS), also recently began recruiting to investigate the efficacy of blocking dihydrotestosterone (DHT), the more potent form of testosterone, which is exclusively abundant in skeletal muscle, via the use of dutasteride, an inhibitor of 5α-reductase (which converts testosterone to DHT), in SBMA patients (21).

It has also been suggested that neuronal degeneration could be associated with transglutaminase-mediated crosslinking of the polyQ AR. These insoluble protein aggregates can inhibit the cell's so-called recycle bin (the proteasome), preventing clearance of the toxic substance and contributing to neuronal cell dysfunction (22). Thus, transglutaminase inhibitors could be beneficial therapeutic agents.

Mutated AR has been shown to sequester CREB-binding protein (CBP), a histone acetyltransferase, which attenuates protein acetylation, modifies gene expression and evokes neuronal toxicity and apoptosis. Correspondingly, restoration of CBP levels and reversal of this hypoacetylation via treatment with deacetylase inhibitors such as vorinostat can rescue neuronal cells from polyQ toxicity (23).

Heat shock proteins (Hsps) have been postulated as targets to abrogate polyQ toxicity, as they play a major role in the intracellular transport of proteins. One such protein is Hsp90, which functions as part of a multichaperone complex that folds, activates and assembles its client proteins. Administration of 17-allylamino-17-demethoxygeldanamycin (17-AAG), a potent Hsp90 inhibitor, has been shown to markedly ameliorate motor impairment in SBMA transgenic mice due to reduced mutant AR (24). On the other hand, overexpression of Hsp70 can inhibit toxic accumulation of polyQ AR and suppress cell death in models of SBMA (25). Thus, pharmacological induction of Hsp70 in the central nervous system via the use of teprenone can ameliorate polyQ-dependent neuromuscular phenotypes (26).

Finally, polyQ ARs have been identified as substrates for cell death proteases or caspases, while mutation of the caspase cleavage site in AR attenuates cell death (27). Therefore, drugs that inhibit caspase-3 activation or prevent proteolytic cleavage could be beneficial for SBMA. A recent publication identified four drugs (digitoxin, nerifolin, peruvoside and suloctidil) that can reduce polyQ-induced cell death by 30-40% (28).

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