Genetics and Molecular Biology, 36, 3, 299-307 (2013)

www.sbg.org.br

Review Article

Histone deacetylase inhibitors as potential treatment for spinal muscular atrophy

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Abstract

Histone acetylation plays an important role in regulation of transcription in eukaryotic cells by promoting a more relaxed chromatin structure necessary for transcriptional activation. Histone deacetylases (HDACs) remove acetyl groups and suppress gene expression. HDAC inhibitors (HDACIs) are a group of small molecules that promote gene transcription by chromatin remodeling and have been extensively studied as potential drugs for treating of spinal muscular atrophy. Various drugs in this class have been studied with regard to their efficacy in increasing the expression of survival of motor neuron (SMN) protein. In this review, we discuss the current literature on this topic and summarize the findings of the main studies in this field.

Keywords: HDACi, molecular therapy, spinal muscular atrophy.

Received: January 1, 2013; Accepted: June 20, 2013.

Introduction

Proximal spinal muscular atrophy (SMA) is a fatal, autosomal recessive pediatric neuromuscular disorder that is characterized by the destruction of α-motor neurons in the anterior horn of the spinal cord. SMA has an estimated incidence of 1/6,000 to 1/10,000 live births, with a carrier frequency of ~1/50 individuals (Burlet*et al.,* 1996; Feldkotter *et al.*, 2002; Kernochan *et al.*, 2005). The criteria for classifying SMA include age of onset and disease progression, based on which SMA patients can be classified into one of four types. Entire gene deletion as well as a variety of intragenic deletions, point mutations and other truncating mutations of survival of motor neuron1 (*SMN1*) on chromosome 5q13that lead to loss of gene function are the cause of SMA (Clermont *et al.*, 1994; Lefebvre *et al.*, 1995; Burglen *et al.*, 1996; Burlet *et al.*, 1996). A highly related homolog of the gene, *SMN2* or centromeric *SMN*, is retained (with a variable copy number) in all SMA patients. The substitution of a C by T at position+6 disrupts a exon splice-enhancing region in exon 7. This change results in most *SMN2* transcripts lacking exon 7 and encodes a truncated protein (Feldkotter *et al.*, 2002; Kernochan *et al.*, 2005).

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SMN2 has, for many years, provided a promising opportunity for correcting SMN deficiency. The fact that *SMN2* produces SMN protein, although at an insufficiently low amount, led investigators to search for ways of increasing the full-length expression of this gene in order to ensure a sufficient level of the protein. Studies in transgenic mice have shown that the insertion of eight copies of human *SMN2* into the mouse genome completely rescued *Smn-/-* mice (*Smn-/-; hSMN2*+/+) from the SMA phenotype (Monani *et al.*, 2003). In humans, a high copy number of *SMN2* may prevent *SMN1*-deficient individuals from manifesting the SMA phenotype (Prior *et al.*, 2004). An increase in full-length SMN protein production through enhanced *SMN2* expression may be achieved through promoter activation, modulation of exon 7 splicing (inclusion of exon 7 in the *SMN2* transcript) or both. Another therapeutic target includes *SMN1* subtle mutations. A subset of SMA patients carrying *SMN1* subtle mutations is susceptible to nonsense-mediated mRNA decay (NMD) (Brichta *et al.*, 2008). In this regard, studies aimed at identifying substances that can stabilize *SMN* mRNA, especially those that express the full-length protein, are of interest.

Various approaches have been proposed as potential means of treating and/or preventing SMA, including: (1) the use of compounds that enhance *SMN2* promoter activity, (2) the use of compounds that modulate *SMN2* splicing, (3) the use of drugs that stabilize *SMN2* mRNA or SMN protein, (4) gene therapy and (5) stem cell therapy (Simic, 2008).

One group of drugs in particular, namely, histone deacetylase(HDAC)inhibitors, has been found to increase *SMN2* promoter activity. Histone acetylation is an important epigenetic mechanism that regulates gene expression. When the N-terminus of core histones is acetylated the corresponding chromatin region is more actively transcribed because of increased accessibility to the DNA. Several drugs in this group have shown promising results in increasing *SMN* promoter activity as will be summarized below.

This article focuses on HDAC inhibitors that target classic HDACs and provides a comprehensive overview of current research on SMA therapy using these inhibitors. Specifically, we will discuss the characteristics and therapeutic potential of valproic acid, phenylbutyrate, benzamide M344, suberoylanilidehydroxamic acid, LBH589, trichostatin A, MS-275, romidepsin, resveratrol, curcumin and epigallocathecin gallate.

HDACs and HDAC inhibitors

Histone remodeling by acetylation and/or deacetylation plays an important role in the transcriptional regulation of eukaryotic cells. Histone acetylation produces a more relaxed chromatin structure that allows transcriptional activation (Kernochan *et al.*, 2005; Riester *et al.*, 2007). This is achieved through the acetylation of lysine residues that imparts a negative charge to the affected amino acid which in turn relaxes the chromatin. In this regard, HDACs are actually "lysinedeacetylases" (Grayson *et al.*, 2010; Xu *et al.*, 2007). HDACs therefore repress transcription through histone deacetylation.

HDACs form a large family of enzymes and have been classified into two groups based on their co-enzyme requirements and sequence similarity to yeast HDACs. These two groups, known as classic HDACs and Sir2-related HDACs (Sirtuins or Class III HDACs), are activated by $Zn^{2+}$and NAD^{+}, respectively. Classic HDACs are subdivided into three smaller classes that include HDAC-I (Ia, Ib and Ic), HDAC-II (IIa and IIb) and HDAC-IV. Each of these smaller classes consists of functional HDAC enzymes (HDAC1 to HDAC11) that are targeted by different HDAC inhibitors (Table 1A,B).Overall, there are 11 classic HDAC enzymes while the Sirtuins contain seven members (Sirt1-Sirt7) (Xu *et al.*, 2007; Nakagawa and Guarente, 2011).

HDAC inhibitors selectively alter gene transcription through chromatin remodeling and by changing the protein structure of transcription factor complexes (Kernochan *et al.*, 2005; Riester *et al.*, 2007). HDAC inhibitors generally consist of three domains: a linker region, a capping group and a metal moeity (Dayangac-Erden *et al.*, 2011).

Valproic acid

Valproic acid (VPA) or Depakene is a Federal Drug Administration (FDA)-approved drug with a terminal half-life ($t_{1/2}$) of 8-10 h in human serum and is frequently used to treat epilepsy, mood disorders and migraine (Brichta *et al.*, 2003). Although VPA is associated with few neurological side effects, hematological and hepatic side effects are well known (Cotariu and Zaidman, 1988; Lackmann, 2004; Tong *et al.*, 2005). VPA increases SMN protein levels through transcriptional activation but also increases the expression of additional serine/arginine (SR)- rich proteins that may have important implications for disorders (including SMA) caused by mutations that result in alternative splicing. While promising results have been obtained *in-vitro*, clinical trials have yielded variable results (Table 2).

Chemical characteristics: VPA is a simple eight-carbon branched fatty acid (carboxylic acid;$C_8H_{14}O_2$) designated as 2-propylpentanoic acid but is also known as dipropylacetic acid.

Phenylbutyrate

Phenyl butyric acid (PBA) or buphenyl is a short-chain fatty acid that has been clinically tested as an anti-cancer drug. In normal tissues, PBA shows little toxicity

Table 1A - Classification of classic histone deacetylases (HDAC).

Class	Subclass	HDAC enzymes	Cellular localization
I	Ia	HDAC1	Nucleus
		HDAC2	Nucleus
	Ib	HDAC3	Nucleus and cytoplasm
	Ic	HDAC8	Nucleus
II	IIa	HDAC4	Nucleus and cytoplasm
		HDAC5	Nucleus and cytoplasm
		HDAC7	Nucleus and cytoplasm
		HDAC9	Nucleus and cytoplasm
	IIb	HDAC6	Nucleus and cytoplasm
		HDAC10	Nucleus and cytoplasm
IV	No subclass	HDAC11	Nucleus and cytoplasm

Table 1B - Histone deacetylase (HDAC) inhibitors and their target enzymes.

Inhibitor	Target HDAC	IC_{50}	Fold increase of full-length SMN2 transcript or SMN protein
VPA	HDAC1, HDAC2, HDAC3	0.7-20 mM	2-4
PBA	HDAC1, HDAC2	16 nM	0.4-2.4
M344	HDAC6	423 nM	3-7
LBH589	Pan HDACs	5-20 nM	10
SAHA	HDAC1, HDAC2, HDAC3, HDAC8, HDAC9	10 nM	5
TSA	HDAC5	1.8 nM	2
MS-275	HDAC1, HDAC2, HDAC3, HDAC9	0.5 μM	Unknown
Romidepsin	HDAC1 HDAC2	36 & 47 nM	5
Resveratrol	HDAC8	650 μM	1.3
Curcumin	HDAC8	25 μM	1.7
EGCG	Unknown	Unknown	1.4

EGCG – epigallocathecin gallate; M344 – benzamide 344; MS-275 – entinostat; PBA – phenylbutyrate; SAHA – suberoylanilidehydroxamic acid;TSA – trichostatin A;VPA – valproic acid.

Table 2 - Summary of studies on valproic acid (VPA) for the treatment of spinal muscular atrophy.

Studies	Country	Study type	Results	Disadvantage
Brichta *et al.* (2003)	Germany	*In vitro* (cell-based); *Ex vivo*	VPA increased SMN protein levels by 2-4 fold after 48 h in fibroblasts cultured from SMA patients and up-regulated SR and SR-like splicing factor;VPA also increased SMN protein levels through transcriptional activation in OHSC cells from rat hippocampus.	Not reported
Sumner *et al.* (2003)	USA	*In vitro* (cell-based)	VPA dose-dependently increased the levels of full-length transcripts (by 147%) more than those of exon 7-containing SMN transcripts (44%).	Not reported
Hahnen *et al.* (2006)	Germany	*In vitro* (cell-based); *Ex vivo*	VPA increased SMN protein levels (by 142%) with no toxicity to rat brain parenchyma at millimolar concentrations and stimulated proteosomal degradation of HDAC2.	Not reported
Hauke *et al.* (2009)	Germany	*In vitro* (cell-based)	VPA showed only moderate effects in response to bypass LT-SMN2 gene silencing in cultured human organotypic hippocampal slice cells(OHSC) and elevated the total SMN2 transcript level but could not significantly bypass LT-SMN2 gene silencing in SMA fibroblasts.	Not reported
Rak *et al.* (2009)	Germany	*In vitro* (cell-based)	VPA elevated SMN expression in neural stem cells and dose-dependently reduced axon length in primary cultures of mouse embryonic motor neurons, although the reduction was not significant. VPA impaired motor neuron survival.	High dose of VPA killed embryonic stem cells
Harahap *et al.* (2011)	Japan	*In vitro* (cell-based)	VPA increased full-length and exon 7-excluding (Δ7) transcript levels in cell lines, modulated splicing factor SF2/ASF expression and decreased hnRNPA1 expression. SMN and SF2/ASF protein levels were increased by 1.5 fold and 1.5-2 fold, respectively, at high VPA concentrations.	Not reported
Brichta *et al.* (2006)	Germany	*In vivo* (pilot trial)	VPA increased the transcript levels of full-length SMN and Δ7 isoform in responder patients but this was not significant when compared to the control and carrier groups. White blood cells were not suitable for studying SMA.	Not reported
Swoboda *et al.* (2009)	USA and Canada	*In vivo* (pilot trial)	VPA was safe and well-tolerated in patients > 2 years old. Carnitine supplementation was needed to decrease the risk of muscle weakness or hepatotoxicity.	Not reported
Piepers *et al.* (2010)	Netherlands	Clinical trial	VPA increased SMN protein levels by up to 20%in SMA patients but this increase was unstable.	No serious adverse effect reported
Swoboda *et al.* (2010)	USA	Clinical trial	VPA had no therapeutic benefit during six months of treatment.	Not reported
Darbar *et al.* (2011)	Brazil	Clinical trial	Improvement in muscle strength and motor abilities were noted, although the benefit was only marginal. VPA was suggested as a potential alternative for alleviating disease progression.	No adverse effects observed

and provides protection against various stimuli. Sodium PBA is a pro-drug that is rapidly metabolized to phenylacetate, a metabolically-active derivative. Phenylacetate conjugates with glutamine via acetylation to form phenylacetylglutamine that is excreted by the kidneys. PBA shows anticancer activity that is generally attributed to its activity as an HDAC inhibitor. Table 3 summarizes studies that have investigated PBA in SMA.

Chemical characteristics: PBA (molecular weight: 186; $C_{10}H_{11}O_2Na$) is known chemically as 4-phenylbutyric acid and is usually supplied as a sodium salt.

Benzamide M344

M344 is a HDAC inhibitor that increases the level of hyperacetylated histone H4 and significantly increases *SMN2* mRNA/protein levels in SMA cells by inducing terminal cell differentiation. M344 shows a three-fold selectivity for inhibition of HDAC6 over HDAC1.Table 4 summarizes studies that have investigated benzamide M344 in SMA.

Chemical characteristics: M344 (N-hydroxyl-7-aminoheptanamide) is a benzamide with the molecular formula $C_{16}H_{25}N_3O_3$.

LBH589

LBH589 (Panobinostat) is a potent putative anticancer drug in numerous cancer cell lines and was given orphan drug status for the treatment of cutaneous T-cell lymphoma (CTCL) by the FDA in 2007. LBH589 is also a novel hydroxamic-acid-derived HDAC inhibitor that is active against all classes of HDACs at low nanomolar con-

Table 3 - Summary of studies on phenylbutyrate for the treatment of spinal muscular atrophy.

Studies	Country	Study type	Results	Disadvantage
Andreassi *et al.* (2004)	Italy	*In vitro* (cell-based)	Phenylbutyrate increased full-length SMN2 transcripts by 50-160% in SMA type I cell and by 80-400% in SMA type II and III cells. Phenylbutyrate was also effective in enhancing SMN protein levels and the number of SMN-containing nuclear structures (gems)*.	Not reported
Dayangac-Erden *et al.* (2008)	Turkey	*In vitro* (cell-based)	Phenylbutyrate did not increase full-length SMN2 transcripts and SMN proteins in EBV-transformed lymphoblastoid cells.	EBV-transformed lymphoblastoid cells are not suitable for this type of study
Hauke *et al.* (2009)	Germany	*In vitro* (cell-based)	Phenylbutyrate showed only moderate effects on bypass LT-SMN2 gene silencing in cultured human organotypichippocampal slice cells (OHSC) and elevated total SMN2 transcript levels.	Not reported
Brahe *et al.* (2005)	Italy	Clinical trial	Phenylbutyrate increased full-length SMN transcript levels by 0.2-2.4 fold in leukocytes from type II and type III SMA patients. Clinical improvement varied markedly from no effect to significant in only six patients.	Short drug half-life (0.8-1 h)
Gonin (2010) (clinicaltrials.gov)	USA	Clinical trial	Clinical trial terminated because of poor compliance to drug administration	Not reported

*The SMN protein is expressed in most tissues and is localized in the cytoplasm and in the nucleus, where it appears concentrated in dot-like structures known as gems.

Table 4 - Summary of studies on benzamide M344 for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Riessland *et al.* (2006)	Germany	*In vitro* (cell-based)	M344 increased FL-SMN2 mRNA levels by restoring the splicing pattern and transcriptional activation of SMN2; there was also an increase in the level of SR and SR-like splicing factors and in the number of nuclear gems. M344 increased the SMN protein levels by 3-7 folds at concentrations of 30-50 μM after 64 h of treatment.	Cytotoxic at > 50μM (MTT assay)
Hahnen *et al.* (2006)	Germany	*In vitro* (cell-based) *Ex vivo*	M344 increased the SMN protein levels in human SMA-affected fibroblasts by up to 168% at 10 μM. In rat OHSC the SMN transcript levels increased by 149% after a 48 h exposure to M344.	Cytotoxic for rat OHSC at > 20 μM (propidium iodide staining)
Hauke *et al.* (2009)	Germany	*In vitro* (cell-based)	M344 increased the total SMN2 transcript levels in human OHSC by up to 188% at 16 μM by bypassing gene silencing.	Not reported

centrations. Table 5 summarizes a study that investigated LBH589 in SMA.

Chemical characteristics: LBH589 (Panobinostat, NVP-LBH589) belongs to the hydroxamate class of inhibitors. The molecular formula is $C_{21}H_{23}N_3O_2$.

Suberoylanilidehydroxamic acid (SAHA)

Suberoylanilidehydroxamic acid (SAHA;zolinza or vorinostat) was initially approved for the treatment of cutaneous T-cell lymphoma (CTCL). Vorinostat, an FDA-approved pan-histone deacetylase inhibitor, is a potentially useful drug for clinical trials in SMA patients. Some of this drugs side-effect includes gastrointestinal symptoms, constitutional symptoms (thrombocytopenia, anemia), taste disorders, pulmonary embolism and anemia. Severe thrombocytopenia and gastrointestinal bleeding have been reported with the concomitant use of zolinza and other HDAC inhibitors, *e.g.*,valproic acid. Table 6 summarizes studies that have investigated SAHA in SMA.

Chemical characteristics: SAHA (N-hydroxy-N'-phenyloctanediamide; $C_{14}H_{20}N_2O_3$) is poorly soluble in water, slightly soluble in ethanol, isopropanol and acetone, freely soluble in dimethyl sulfoxide and insoluble in methylene chloride.

Trichostatin A (TSA)

Trichostatin A (TSA), originally developed as an antifungal drug, is a member of a large class of HDAC inhibitors that has a broad spectrum of epigenetic activities. TSA selectively inhibits class I and II mammalian HDAC. TSA alters gene expression by interfering with the removal of acetyl groups from histones by HDAC and therefore alters the ability of DNA transcription factors to access the DNA within chromatin. TSA is harmful by inhalation and is irritating to the eyes, respiratory system and skin. Table 7 summarizes the studies on TSA in SMA.

Chemical characteristics: TSA (7-[4-(dimethylamino)phenyl]-N-hydroxy-4,6R-dimethyl-7-oxo-2E,4E-heptadienamide; $C_{17}H_{22}N_2O_3$) is extracted from *Streptomyces platensis* and is soluble in ethanol and dimethylsulfoxide (DMSO).

Table 5 - Summary of a study on LBH589for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Garbes *et al.* (2009)	Germany	*In vitro* (cell-based)	The SMN protein level increased by up to 10 fold at 400 nM LBH589 after a 64-h exposure. A number of gems and a stable increase in SMN protein were also observed.	No cytotoxic effects at up to 500 nM

Table 6 - Summary of studies on SAHA for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Riessland *et al.* (2006)	Germany	*Ex vivo*	SAHA elevated SMN expression in spinal cord and muscle, improved motor abilities and increased body weight of SMA mice.	Not reported
Hahnen *et al.* (2006)	Germany	*In vitro* (cell-based) *Ex vivo*	SAHA increased full-length SMN2 transcript levels in SMA-affected human fibroblasts, rat OHSC and rat glioma cells by up to 296%, 167% and 176%, respectively.	SAHA caused no detectable toxicity in OHSC up to 80 μM
Hauke *et al.* (2009)	Germany, Australia	*In vitro* (cell-based)	SAHA bypassed LT-SMN2 gene silencing in SMA fibroblasts and induced a ~25-fold increase of LT-SMN2 (long transcript; started at -296) and a 5-fold increase of total SMN2 transcript levels at 30 μM. In human OHSC, SAHA increased LT-SMN and total SMN protein levels by up to 219% at 32 μM after 48 h.	Not reported

Table 7 - Summary of studies on TSA for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Avila *et al.* (2007)	USA, Italy	*In vitro* (cell--based) *Ex vivo*	TSA induced SMN2 promoter activation by approximately two fold after 2-4 h of exposure. TSA markedly improved motor performance, attenuated weight loss, increased survival and improved the pathology of the motor unit in SMA mice	One-quarter of SMA mice showed no response to TSA treatment
Narver *et al.* (2008)	USA	*Ex vivo*	TSA improved short-term function and produced long-lasting stabilization of the SMA motor unit. In affected mice treated with TSA and a dietary supplementation the median survival time increased by up to 38 days (170%) as compared to non-treated mice.	Tissue necrosis

Entinostat (MS-275)

Entinostat(MS-275;n-2-aminophenyl-4-n-pyridine-3 -ylmethoxycarbonylaminomethyl-benzamide), is a cell-permeable benzamide analog that inhibits HDAC and induces differentiation and transcription of growth factor βII receptor (TβRII), in addition to inhibiting the proliferation of human breast cancer cells. Table 8 summarizes studies that have investigated Entinostat in SMA.

Chemical characteristics: The molecular formula of Entinostat is $C_{21}H_{20}N_4O_3$.

Romidepsin

Romidepsin (Istodex or FK228), an HDAC inhibitor from *Chromobacterium violaceum*, is a bicyclic depsipeptide. Romidepsin is indicated for the treatment of CTCL in patients who have received at least one prior systemic therapy. Romidepsin shows hematologic and non-hematologic toxicity at high doses. Table 9 summarizes a study that investigated the usefulness of romidepsinin SMA.

Chemical characteristics: Romidepsin is described chemically as (1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis(1 methylethyl)-2-oxa-12,13-dithia-5,8,20,23-tetra azabicyclo[8.7.6]tricos-16ene-3,6,9,19,22-pentone with the molecular formula $C_{24}H_{36}N_4O_6S_2$.

Resveratrol

Resveratrol (Kojo-Kon, Phytoalexin, Phytoestrogen and SRT-501) is a chemical found in red wine, red grape skins, purple grape juice, mulberries and in smaller amounts in peanuts. Resveratrol is used against hardening of the arteries (atherosclerosis), high cholesterol and for the prevention of cancer. Resveratrol may increase the risk of bleeding. Table 10 summarizes studies that have investigated resveratrol in SMA.

Chemical characteristics: Resveratrol, a polyphenolic compound ((E)-resveratrol (3,5,4$^{'}$-trihydroxy-trans-stilbene)), belongs to the stilbene class of molecules and is classified as anti-cancer, antioxidant and enzyme inhibitor. The molecular formula is $C_{14}H_{12}O_3$.

Curcumin

Curcumin is a mixture of compounds derived from the curry spice turmeric and is used as an herbal supplement. Curcumin (diferuloylmethane) is a new HDAC inhibitor that inhibits the expression of class I HDACs (HDAC1, HDAC3and HDAC8). Curcumin possesses a spectrum of pharmacological properties that have been attributed primarily to its inhibition of metabolic enzymes. Curcumin has been alleged to have antioxidant, antiviral, anti-inflammatory and anticancer activities, as well as cholesterol-lowering effects.

Chemical characteristics: Curcumin, a natural polyphenol and the major component of turmeric has the molecular formula $C_{21}H_{20}O_6$.

Epigallocatechin gallate

Epigallocatechin gallate (EGCG; Sinecatechins or Veregen), a partially purified fraction obtained from a water extract of green tea (*Camellia sinensis*) leaves, is used topically and is a potent antioxidant.Table 11 summarizes studies that have tested curcumin and EGCG in SMA.

Chemical characteristics: The molecular formula for epigallocatechin gallate is $C_{15}H_{14}O_7$.

Discussion

Eight of the 11 known HDACs were inhibited by the compounds reviewed here; HDAC4, HDAC7 and HDAC10 were not inhibited by any of the compounds. As shown in Table 1B, the fold increase of full-length *SMN2* transcripts or SMN protein varied considerably (from 0.4 to 10).

Five compounds (VPA, M344, resveratrol, EGCG and curcumin) acted by two mechanisms, namely, (1) by increasing the overall *SMN2* expression through inhibition

Table 8 - Summary of studies on MS-275 for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Hahnen *et al.* (2006)	Germany	*In vitro* (cell-based) *Ex vivo*	MS-275 did not increase SMN expression in mouse OHSC and did not activate the SMN2 gene in human fibroblast-derived cells from SMA patients.	MS-275 had no apparent impact on SMN expression in mouse OHSC and human fibroblasts
Hauke *et al.* (2009)	Germany, Australia	*In vitro* (cell-based)	MS-275 had a moderate effect on bypass LT-SMN2 gene silencing in SMA fibroblasts and human OHSC. MS-275 caused a moderate increase in gene expression.	Not reported

Table 9 - Summary of a study on romidepsin for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Hauke *et al.* (2009)	Germany, Australia	*In vitro* (cell-based)	Romidepsin bypassed LT-SMN2 gene silencing and resulted in a five-fold increase in the total SMN2 transcript level in human fibroblasts.	Not reported

Table 10 - Summary of studies on resveratrol for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Sakla and Lorson (2008)	USA	*In vitro* (cell-based)	Resveratrol elevated SMN2-luciferase expression by six fold and increased the exon 7-inclusion by 1.4 fold in a luciferase assay. These effects translated into only a one-fold increase in the full-length SMN2 transcript level.	Not reported
Dayangac-Erden *et al.* (2009)	Turkey	*In vitro* (cell-based)	Resveratrol increased the full-length SMN2 mRNA and protein levels by 1.3-fold.	Not reported

Table 11 - Summary of studies on curcumin and EGCG for the treatment of spinal muscular atrophy.

Study	Country	Study type	Results	Disadvantage
Sakla and Lorson (2008)	USA	*In vitro* (cell-based)	Polyphenolic compounds (curcumin and EGCG) increased the efficiency of *SMN2* exon 7 inclusions. There was increase in SMN protein levels and number of activated gems after exposure to these compounds. Total SMN protein elevation was 1.4 fold after exposure to EGCG.	Not reported
Dayangac-Erden *et al.* (2011)	Turkey	*In vitro* (cell-based)	Curcumin increased FL-SMN mRNA level significantly by up to 1.7 fold and caused a concentration-dependent increase in exon 7 inclusions.	No Reported

of targeted HDACs and (2) by increasing the incorporation of exon 7 into the *SMN2* transcripts through the activation of splicing factors. However, the latter three compounds induced only a minimal increase in the total *SMN2* transcript level. Nevertheless, these compounds may still have useful chemical properties because they are derived from natural products and show few or no adverse effects. In this regard, *insilico* analyses may be helpful in optimizing the design of molecules with greater effect on *SMN2* while retaining their safety.

In addition to HDAC inhibition, an increase in the overall *SMN2* transcript level can also be achieved by de-methylation of the *SMN2* gene. An increase in *SMN2* expression through de-methylation, *i.e.*, bypassing *SMN2* gene silencing, was recently suggested for SAHA, MS275 and Romidepsin (Hauke*et al.,* 2009), and indicated that these three drugs to have a double mechanism of action in addition to inhibiting targeted HDACs. However, de-methylation contributed to only 5% of the total increase in full-length transcripts.

In contrast, inhibition of HDAC6 by LBH-589 and M344 resulted in the highest fold increase of full-length transcripts, even when compared to inhibition of multiple HDACs. Li *et al.* (2013) indicated that, unlike other deacetylases, HDAC6 has a unique substrate specificity for non-histone proteins. This diversity of functions for HDAC6 suggests that this enzyme could be a potential therapeutic target for the treatment of a wide range of diseases. In this regard, finding an inhibitor of HDAC6 may help in the search for a potent *SMN2* expression activator. It would also be worthwhile to study the effects of currently known HDAC6 inhibitors in SMA cell lines. Once the structure of HDAC6 is known molecular docking strategies may be used to identify natural or synthetic inhibitors of this enzyme.

Only two of the HDAC inhibitors discussed here (PBA and VPA) have entered clinical trials for human use. The results of these clinical trials have varied considerably and a systematic review of potential drugs for treating SMA found that none of them, including HDAC inhibitors, were efficacious in treating this condition (Wadman *et al.*, 2012a,b).

Conclusion

We have summarized various studies that have examined the usefulness of HDAC inhibitors for treating SMA. Naturally-derived HDAC inhibitors (also summarized here) are less toxic but also show less therapeutic promise. Given the therapeutic potential of HDAC inhibitors and their theoretical mechanism of action, a search for further inhibitors is warranted in an effort to identify molecules with suitable properties (high blood-brain barrier penetration and minimal/tolerable adverse effects) that can be used to correct the molecular pathology of SMA.

Acknowledgments

This work was supported by Universiti Sains Malaysia Research University grants 1001/PPSP/812072 and 1001/PPSP/812048 to THS. JM is the recipient of a Universiti Sains Malaysia graduate assistant scholarship.

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Associate Editor: Maria Rita Passos-Bueno