Histone deacetylase inhibitors as potential treatment for spinal muscular atrophy

Jafar Mohseni1, Z.A.M.H. Zabidi-Hussin2 and Teguh Haryo Sasongko1

1Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia.
2Department of Pediatrics, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia.

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 5q13 that 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).

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 epigallocatechin 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 Zn2+ and NAD+, respectively. Classic HDACs are subdivided into three smaller classes that include HDAC-I (Ia, Ib and Ic), HDAC-II (Ila 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 moiety (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 (t1/2) 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; C8H14O2) 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 anticancer 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    | Ila      | 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<sub>50</sub> | 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 – epigallocatechin 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 upregulated 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 htrRNA1 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. | 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; C₁₀H₁₁O₂Na) 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-aminohexanamide) is a benzamide with the molecular formula C₁₆H₂₅N₃O₃.

**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.   | Italy   | In vitro   | 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)¹. | Not reported                                      |
| Dayangac-Erden et al. | Turkey | In vitro   | 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.       | Germany | In vitro   | Phenylbutyrate showed only moderate effects on bypass LT-SMN2 gene silencing in cultured human organotypic hippocampal slice cells (OHSC) and elevated total SMN2 transcript levels. | Not reported                                      |
| Brahe et al.       | 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)       | USA     | Clinical trial | Clinical trial terminated because of poor compliance to drug administration | Not reported                                      |

¹The 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.   | Germany | In vitro   | 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.      | Germany | In vitro   | 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.       | Germany | In vitro   | 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$_{3}$O$_{2}$.

**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$_{2}$O$_{3}$) 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-(dimethylaminophenyl]-N-hydroxy-4,6R-dimethyl-7-oxo-2E,4E-heptadienamide; C$_{17}$H$_{22}$N$_{2}$O$_{3}$) is extracted from *Streptomyces platensis* and is soluble in ethanol and dimethylsulfoxide (DMSO).

---

**Table 5** - Summary of a study on LBH589 for 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 |
| Narvet *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-ylmethoxy carbonylaminomethyl-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 C21H20N4O3.

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 romidepsin in 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-tetraazabicyclo[8.7.6]tricos-16ene-3,6,9,19,22-pentone with the molecular formula C24H36N4O6S2.

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 C14H12O3.

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, HDAC3 and 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 C21H20O6.

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 C15H14O7.

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

| Study     | Country        | Study type            | Results                                                                 | Disadvantage                                      |
|-----------|----------------|-----------------------|-------------------------------------------------------------------------|---------------------------------------------------|
| Hahnen et al. (2006) | Germany | In vitro (cell-based) | 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                                      |

| 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                                      |
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.

References

Andreassi C, Angelozzi C, Tiziano FD, Vitali T, De Vincenzi E, Boninsegna A, Villanova M, Bertini E, Pini A, Neri G, et al. (2004) Phenylbutyrate increases SMN expression in vitro:
Relevance for treatment of spinal muscular atrophy. Eur J Hum Genet 12:59-65.

Avila AM, Burnett BG, Taye AA, Gabanella F, Knight MA, Hartenstein P, Cizman Z, Di Prospero NA, Pellizzoni L, Fischbeck KH, et al. (2007) Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. J Clin Invest 117:659-671.

Brahe C, Vitali T, Tiziano FD, Angelozzi C, Pinto AM, Borgo F, Moscato U, Bertini E, Merceri E and Neri G (2005) Phenylbutyrate increases SMN gene expression in spinal muscular atrophy patients. Eur J Hum Genet 13:256-259.

Brichta L, Hofmann Y, Hahnen E, Siebzehnrubl FA, Raschke H, Blumcke I, Eyupoglu IY and Wirth B (2003) Valproic acid increases the SMN2 protein level: A well-known drug as a potential therapy for spinal muscular atrophy. Hum Mol Genet 12:2481-2489.

Brichta L, Holker I, Haug K, Klockgether T and Wirth B (2006) In vivo activation of SMN in spinal muscular atrophy carriers and patients treated with valproate. Ann Neurol 59:970-975.

Brichta L, Garbes L, Jedrzejowska M, Grellscheid SN, Holker I, Zimmermann K and Wirth B (2008) Nonsense-mediated messenger RNA decay of survival motor neuron 1 causes spinal muscular atrophy. Hum Genet 123:141-153.

Burglen L, Lefebvre S, Clermont O, Burlet P, Viollet L, Cruaud C, Munnich A and Melki J (1996) Structure and organization of the human survival motor neurone (SMN) gene. Genomics 32:479-482.

Burlet P, Burglen L, Clermont O, Lefebvre S, Viollet L, Munnich A and Melki J (1996) Large scale deletions of the 5q13 region are specific to Werdnig-Hoffmann disease. J Med Genet 33:281-283.

Clermont O, Burlet P, Burglen L, Lefebvre S, Pascal F, McPherson J, Wasmuth JJ, Cohen D, Le Paslier D, Weissenbach J, et al. (1994) Use of genetic and physical mapping to locate the spinal muscular atrophy locus between two new highly polymorphic DNA markers. Am J Hum Genet 54:687-694.

Darbar IA, Plaggert PG, Resende MBD, Zanoteli E and Reed UC (2011) Evaluation of muscle strength and motor abilities in children with type II and III spinal muscle atrophy treated with valproic acid. BMC Neurology 11:6.

Dayangac-Erden D, Topaloglu and Erdem-Yurter H (2008) A preliminary report on spinal muscular atrophy lymphoblastoid cell lines: Are they an appropriate tool for drug screening? Adv Ther 25:274-279.

Dayangac-Erden D, Bora-Tara G, Ayhan P, Kocaefe C, Dalkara S, Yelek IE, Erdem-Yurter H and Demir AS (2009) Histone deacetylase inhibition activity and molecular docking of (E)-resveratrol: Its therapeutic potential in spinal muscular atrophy. Chem Biol Drug Des73:355-364.

Dayangac-Erden D, Bora-Tatar G, Dalkara S, Demir AS and Erdem-Yurter H (2011) Carboxylic acid derivatives of histone deacetylase inhibitors induce full length SMN2 transcripts: A promising target for spinal muscular atrophy therapies. Arch Med Sci 7:230-234.

Feldkotter M, Schwarzer V, Wirth R, Wienker TF and Wirth B (2002) Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: Fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. Am J Hum Genet 70:358-368.

Garbes L, Riessland M, Holker I, Heller R, Hauke J, Trankle C, Coras R, Blumcke I, Hahnen E and Wirth B (2009) LBH589 induces up to 10-fold SMN protein levels by several independent mechanisms and is effective even in cells from SMA patients non-responsive to valproate. Hum Mol Genet 18:3645-3658.

Grayson DR, Kundakovic M and Sharma RP (2010) Is there a future for histone deacetylase inhibitors in the pharmacotherapy of psychiatric disorders? Mol Pharmacol 77:126-135.

Hahnen E, Eyupoglu IY, Brichta L, Haastert K, Trankle C, Siebzehnrubl FA, Riessland M, Holker I, Claus P, Romstock J, et al. (2006) In vitro and ex vivo evaluation of second-generation histone deacetylase inhibitors for the treatment of spinal muscular atrophy. J Neurochem 98:193-202.

Harahap ISK, Saito T, San LP, Sasaki N, Gunadi, Nurputra DPK, Yusoff S, Yamamoto T, Morikawa S, Nishimura N, et al. (2011) Valproic acid increases SMN2 expression and modulates SF2/ASF and hnRNPA1 expression in SMA fibroblast cell lines. Brain Dev 34:213-222.

Hauke J, Riessland M, Lunke S, Eyupoglu IY, Blumcke I, El-Osta A, Wirth B and Hahnen E (2009) Survival motor neuron gene 2 silencing by DNA methylation correlates with spinal muscular atrophy disease severity and can be bypassed by histone deacetylation inhibition. Hum Mol Genet 18:304-317.

Kernochan LE, Russo ML, Woodling NS, Huynh TN, Avila AM, Fischbeck KH and Sumner CJ (2005) The role of histone acetylation in SMN gene expression. Hum Mol Genet 14:1171-1182.

Kissel JT, Scott CB, Reyna SP, Crawford TO, Simard LR, Kroschell KJ, Acasgi G, Elsheik B, Schroth MK, D’Anjou G, et al. (2011) SMA CARNIVAL Trial Part II: A prospective, single-armed trial of L-carnitine and valproic acid in ambulatory children with spinal muscular atrophy. PLoS One 6:e21296.

Lackmann GM (2004) Valproic acid-induced thrombocytopenia and hepatotoxicity: Discontinuation of treatment? Pharmacology 70:57-58.

Lefebvre S, Burglen L, Rebollet S, Clermont O, Burlet P, Viollet L, Benichou B, Cruaud C, Millasseau P, Zeviani M, et al. (1995) Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80:155-165.

Li Y, Shin D and Kwon SH (2013) Histone deacetylase 6 plays a role as a distinc regulator of diverse cellular processes. FEBS J 280:775-793.

Monani UR, Pastore MT, Gavrilina TO, Jablonka S, Le TT, Andreassi A, DiCocco JM, Lorson C, Androphy EJ, Sendtner M, et al. (2003) A transgene carrying an A2G missense mutation in the SMN gene modulates phenotypic severity in mice with severe (type I) spinal muscular atrophy. J Cell Biol 160:41-52.

Nakagawa T and Guarente L (2011) Sirtuins at a glance. J Cell Sci 124:833-838.

Naver HL, Kong L, Burnett BG, Cho DW, Bosch-Marce M, Taye AA, Eckhaus MA and Sumner CJ (2008) Sustained improvement of spinal muscular atrophy mice treated with trichostatin A plus nutrition. Ann Neurol 64:465-470.

Piepers S, Cobben JM, Soodar P, Jansen MD, Wadman RI, Meester-Delver A, Poll-The BT, Lennmink HH, Wokke JH, van der Pol WL, et al. (2010) Quantification of SMN protein in leucocytes from spinal muscular atrophy patients: Effects of treatment with valproic acid. J Neurol Neurosurg Psychiatry 82:850-852.
Prior TW, Swoboda KJ, Scott HD and Hejmanowski AQ (2004) Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. Am J Med Genet 130A:307-10.

Rak K, Lechner BD, Schneider C, Drexl H, Sendtner M and Jablonka S (2009) Valproic acid blocks excitability in SMA type I mouse motor neurons. Neurobiol Dis 36:477-487.

Riessland M, Brichta L, Hahnen E and Wirth B (2006) The benzamide M344, a novel histone deacetylase inhibitor, significantly increases SMN2 RNA/protein levels in spinal muscular atrophy cells. Hum Genet 120:101-110.

Riessland M, Ackermann B, Forster A, Jakubik M, Hauke J, Garbes L, Fritzscbe I, Mende Y, Blumcke I, Hahnen E, et al. (2010) SAHA ameliorates the SMA phenotype in two mouse models for spinal muscular atrophy. Hum Mol Genet 19:1492-1506.

Riester D, Hildmann C and Schwienhorst A (2007) Histone deacetylase inhibitors - Turning epigenic mechanisms of gene regulation into tools of therapeutic intervention in malignant and other diseases. Appl Microbiol Biotechnol 75:499-514.

Sakla M, Sand Lorson CL (2008) Induction of full-length survival motor neuron by polyphenol botanical compounds. Hum Genet 122:635-643.

Simic G (2008) Pathogenesis of proximal autosomal recessive spinal muscular atrophy. Acta Neuropathol 116:223-234.

Sumner TN, Markowitz JA, Perhac JS, Hill B, Coovert DD, Schussler K, Chen X, Jarecki J, Burghes AH, et al. (2003) Valproic acid increases SMN levels in spinal muscular atrophy patient cells. Ann Neurol 54:647-654.

Swoboda KJ, Scott CB, Reyna SP, Prior TW, LaSalle B, Sorenson SL, Wood J, Acsadi G, Crawford TO, Kissel JT, et al. (2009) Phase II open label study of valproic acid in spinal muscular atrophy. PLoS One 4:e5268.

Swoboda KJ, Scott CB, Crawford TO, Simard LR, Reyna SP, Krosschell KJ, Acsadi G, Elsheik B, Schroth MK, D’Anjou G, et al. (2010) SMA CARNI-VAL Trial Part I: Double-blind, randomized, placebo-controlled trial of L-carnitine and valproic acid in spinal muscular atrophy. PLoS One 5:e12140.

Tong V, Teng XW, Chang TK and Abbott FS (2005) Valproic acid II: Effects on oxidative stress, mitochondrial membrane potential, and cytotoxicity in glutathione-depleted rat hepatocytes. Toxicol Sci 86:436-443.

Wadman RI, Bosboom WM, van den Berg LH, Wokke JH, Innaccone ST and Vrancken AF (2012a) Drug treatment for spinal muscular atrophy type I. Cochrane Database Syst Rev CD006281.

Wadman RI, Bosboom WM, van den Berg LH, Wokke JH, Innaccone ST and Vrancken AF (2012b) Drug treatment for spinal muscular atrophy types II and III. Cochrane Database Syst Rev CD006282.

Xu WS, Parmigiani RB and Marks PA (2007) Histone deacetylase inhibitors: Molecular mechanisms of action. Oncogene 26:5541-5552.

Internet Resources

ClinicalTrials.gov, http://www.clinicaltrials.gov/ (22 December, 2012).

PubChem Substance database, http://www.ncbi.nlm.nih.gov/pcsubstance (22 December, 2012).

Selleck Chemicals website, http://www.selleckchem.com (24 April, 2013).

Associate Editor: Maria Rita Passos-Bueno

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.