Benefits of histone deacetylase inhibitors for acute brain injury; a systematic review of animal studies

Claire L. Gibson* and Sean P. Murphy**
*School of Psychology, University of Leicester, Leicester, UK
**Department of Neurological Surgery, University of Washington School of Medicine, Seattle, WA, USA

Abstract

Drugs that inhibit histone deacetylase (HDAC) activities have enormous potential as neuroprotective agents. We performed a systematic review of controlled animal studies that administered known inhibitors of the zinc-dependent HDACs before and/or after acute cerebral injury and assessed anatomic/functional outcomes. Relevant studies were found by searching PubMed, Embase and Web of Science. From more than 100 identified publications, those data meeting specific criteria were analyzed using the Cochrane Review Manager software. A beneficial effect of administering HDAC inhibitors was seen in studies involving cerebral ischemia or non-ischemic models of acute cerebral injury. Specific studies assessed efficacy when drug was administered up to 14 days prior to, and 14 days following, the onset of cerebral injury. This systematic review provides objective evidence of a neuroprotective role for drugs that inhibit HDACs and highlights particular areas that require further experimental investigation.

Keywords
CNS injury; systematic review; HDAC; mouse; rat

Inhibitors of histone deacetylases (HDACs) promote the demise of tumor cells and are of significant interest in the treatment of various cancers (Minucci and Pelicci, 2006; Marks and Xu, 2009; Prince et al., 2009). These same drugs promote the ‘neuronal’ differentiation of neural progenitor cells (Montgomery et al., 2009; Lee and Lee, 2010) and sustain mature neurons and their processes in the face of a variety of insults (Chuang et al., 2009; Langley et al., 2009), generating considerable interest in their potential for the clinical treatment of acute brain injury and for human neurodegenerative conditions (Haberland et al., 2009; Thomas, 2009).

Acetylation is a key post-translational modification of proteins and responsible for regulating critical intracellular pathways. Protein acetylation is the transfer of an acetyl moiety from acetyl-coenzyme A to the ε-amo group of a lysine residue. The acetylation of lysine is reversible and controlled by the opposing actions of histone acetyltransferase (HAT) and HDACs. Although histones represent the most thoroughly studied substrates for these enzymes, the activity of diverse non-histone proteins are also modified by HATs and HDACs, including transcription factors such as p53 and signal transduction mediators (Haberland et al., 2009; Marks and Xu, 2009). The HDACs mainly function as...
transcriptional co-repressors, removing acetyl groups from histones in nucleosomes to promote chromatin condensation and gene repression. Conversely, HATs add acetyl groups to histones, reducing chromatin compaction and enhancing the accessibility of DNA to transcription factors and transcriptional co-activators (Sweatt, 2009). Predictably, HDACs regulate fundamental cellular events including the cell cycle, differentiation, and apoptosis.

The HDACs divide into two categories: “zinc-dependent” and “NAD-dependent” (the sirtuins). The zinc-dependent HDAC family members are Class I (HDAC 1-3, 8), Class II (HDAC 4-7, 9 and 10) or Class IV (HDAC 11), based upon sequence similarity. Class I are found mostly within the nucleus whereas Class II members shuttle between nucleus and cytoplasm. Roles for a particular HDAC in brain maturation and other specific functions, and also in the response to injury, are beginning to become evident (Guan et al., 2009; Kramer, 2009; Montgomery et al., 2009; Rivieccio et al., 2009; Kim et al., 2010). A number of drugs with HDAC inhibitory activity appear to protect neurons against acute injury (Langley et al., 2009). Most of these drugs broadly inhibit Class I and Class II HDAC activities and are generally not specific with regard to the proteins they affect. To objectively assess the animal data, here we report the first systematic review and meta-analysis of the use of inhibitors of the zinc-dependent HDACs in experimental stroke and head injury models. Our aim was to obtain an overall impression of the efficacy of HDAC inhibitors in animal studies and to identify where additional experimental investigation is required. Numerous, recent narrative reviews very adequately discuss HDAC targets, the mechanisms by which hyperacetylation may confer neuroprotection, and cell type specific expression in the CNS (Chuang et al., 2009; Haberland et al., 2009; Langley et al., 2009; Thomas, 2009).

Experimental procedures

Study identification

Experimental (non-human) studies assessing the effects of exogenously applied inhibitors of HDACs in models of acute brain injury (i.e. focal/global ischemia, TBI, experimental cerebral haemorrhage) were identified from PubMed, Embase, and Web of Science by searching for all published articles up to the end of March, 2010. The earliest study included for analysis was that of Ren et al. (2004). Search keywords included HDAC inhibitor and one of the following: cerebral, brain injury, ischemia, stroke, traumatic, or cerebrovascular. Further literature searches were conducted substituting the general term HDAC inhibitor for the most common specific ones, such as ‘valproic acid’, ‘sodium butyrate’ and ‘Trichostatin A’. Studies involving the sirtuins were not included. Additional publications were identified from reference lists of all identified mss. and review articles. Pre-specified exclusion criteria were used to aid selection and prevent bias, and studies were only included if the following were met; (i) experimental cerebral injury was induced, (ii) an HDAC inhibitor was exogenously applied, (iii) no other potential neuroprotective agents were additionally administered (i.e. confounded), (iv) measurements on infarct size or functional outcome were performed, and (v) there was a control group.

Data extraction

Summary data on total infarct size, measured as volume or area (mm\(^3\), percentage of normal brain, or mm\(^2\)), were extracted. In all included studies, data for lesion volume were presented as having been corrected for edema. In two studies, data were included for % degenerating neurons assessed by FluoroJade immunohistochemistry (Zhang et al. 2008) and % edema volume (Sinn et al. 2007). In addition, functional outcome data were extracted and these included: (i) Morris Water Maze performance (latency to escape platform), (ii) Rotarod performance (time spent in seconds or percentage compared to baseline), (iii)
Neurological 4-point or 8-point scales (low scores indicate a better outcome), and (iv) a limb placement test. A comparison (C) was defined as the assessment of outcome in drug and control groups following treatment with an administered dose of the HDAC inhibitor, starting at a stated time before/after the onset of cerebral injury. For each comparison, data were extracted for mean outcome, standard deviation (SD) and the number of animals per group. If published studies (S) used multiple groups, for example to assess dose-response relationships, then data from each group were individually extracted. Occasionally, numerical data were not reported in the text and these were extracted from enlarged, photocopied graphs.

The methodological quality of each study was determined using an eight-point scale, as previously described (Gibson et al. 2006, 2008; England et al. 2009). One point was given for written evidence of the following criteria: presence of randomization, monitoring of physiological parameters (not just maintenance), assessment of dose-response relationship, assessment of optimal time window, masked outcome measurement, assessment of outcome at days 1-3, assessment of outcome at days 1-30, and combined measurement of lesion volume and functional outcome.

Data analysis

The data were analysed as forest plots using Cochrane Review Manager (version 4.2), just as in previous animal meta-analyses (Gibson et al. 2008; England et al. 2009). The effect of the HDAC inhibitor, as compared with control, on lesion volume or functional outcome was assessed using the standardized mean difference (SMD); here, the difference in effect between the HDAC inhibitor and control group is divided by the total SD. This allows comparisons to be made if different methods of measurement or different animal species have been used. These estimates were pooled using the DerSimonian and Laird (1986) random effects model, which takes into account any statistical heterogeneity found between studies. Prior to analysis, data were grouped by (i) model type (i.e. ischemia or other) and (ii) outcome measure (i.e. lesion volume or functional outcome). To examine the effects of study characteristics and potential sources of heterogeneity on outcome, stratified meta-analyses were performed with experiments grouped according to: (i) quality score; (ii) type of HDAC inhibitor, and (iii) timing of HDAC inhibitor administration in relation to the onset of brain injury. Studies were weighted by sample size and the results are expressed as SMD with 95% confidence intervals (CI). The significance level was set at $\alpha = 0.05$ and $p$ values of $< 0.05$ from meta-analyses were considered to be significant.

Results

Design of studies

While our various literature searches identified 101 potential studies, a large number of these were subsequently excluded for the reasons given in Fig. 1. The characteristics of the included studies are reported in Table 1. All of them reported the effect of exogenously administered HDAC inhibitor versus control (i.e. vehicle) on histological damage (lesion volume or % degenerating neurons), or on functional outcome following acute cerebral injury. These studies represent outcomes from 10 independent research groups (three studies from the same laboratory, the remaining nine from separate research groups). Within the 12 studies, data from a total of 582 experimental subjects were included for analysis.

The majority of studies employed a model of focal ischemia; 4 used permanent ischemia (Faraco et al. 2006; Kim et al. 2007, 2009; Langley et al. 2008) and 2 used transient ischemia (Ren et al. 2004; Yildrim et al. 2008). Other models included were experimental intracerebral hemorrhage (Sinn et al. 2007), global ischemia (Kabakus et al. 2005; Qi et al. 2005).
2004) and TBI (Dash et al. 2009; Shein et al. 2009; Zhang et al. 2008). Six of the included studies used Wistar or Sprague-Dawley rats, and the remainder used a variety of mouse strains (C57, Sabra, 129/SV). All studies used males. The HDAC inhibitors were administered either intra-peritoneally or subcutaneously, with first dose administration ranging from 14 days before or 7 days after injury, although the majority of studies first administered a HDAC inhibitor from between one hour before and two hours following injury onset.

In terms of outcome measures, histologic damage was indicated by lesion volume (mm$^3$), percentage of ipsilateral non-ischemic total/region or percentage degenerating neurones. Functional outcome was reported by ‘escape to platform’ latency (i.e. Morris Water Maze performance), time spent on rotarod, a neurological score (4- or 8-point scale), or limb placement function. In terms of the type of HDAC inhibitor, five studies administered sodium butyrate (SB), four studies administered valproic acid (VPA), and two studies administered trichostatin A (TSA). Each of the following drugs was used in an individual study; SAHA (Faraco et al. 2006), ITF2357 (Shein et al. 2009), and DMA-PB (Zhang et al. 2008). The positive Egger’s test of asymmetry (Fig 2) for studies utilising models of focal ischemia ($p < 0.001$) or other models of acute cerebral injury ($p < 0.001$) could imply the presence of publication bias (Egger et al. 1997).

Reported study quality

The median quality rating for all included studies was 4 out of 8 (range 2-5). The median quality rating was the same when studies were grouped separately into ‘ischemia’ or ‘other’ models of acute cerebral injury, which is similar to the median quality rating reported by other systematic reviews examining models of acute cerebral injury (England et al. 2009; Gibson et al. 2009). Few of the included studies reported randomization (Faraco et al. 2006; Kabakus et al. 2005) and the use of blinded outcome assessment (Kabakus et al. 2005; Kim et al. 2007, 2009; Ren et al. 2004; Yildrim et al. 2008). Although variable in terms of HDAC inhibitor dosing regimen, five studies specifically addressed dose response (Faraco et al. 2006; Kabakus et al. 2005; Kim et al. 2007; Qi et al. 2004; Zhang et al. 2008) and six studies investigated the optimal time window of administration (Dash et al. 2009; Kim et al. 2007; Langley et al. 2008; Qi et al. 2004; Shein et al. 2009; Yildrim et al. 2008). The majority assessed outcome at days 1-3, with five studies assessing outcome at days 1-30 (Dash et al. 2009; Kabakus et al. 2005; Kim et al. 2009; Shein et al. 2009; Sinn et al. 2007) and six studies combining assessment of histological damage with functional outcome (Kim et al. 2007; Qi et al. 2004; Ren et al. 2004; Shein et al. 2009; Sinn et al. 2007; Yildrim et al. 2008). A beneficial effect of administering the HDAC inhibitor increased with improving study quality score (Fig.3) in those studies where this was 3 or more.

Type of model

The effects of drug on lesion volume and functional outcome were analysed when studies were separated according to whether they used a model of cerebral ischemia or other type of acute cerebral injury (Fig. 4). Following cerebral ischemia, HDAC inhibition had a beneficial effect on both lesion volume (-3.23, -4.26 to -2.20, $p < 0.001$) and functional outcome (-2.15, -2.82 to -1.47, $p < 0.001$). Likewise, the administration of drug in those studies utilising non-ischemia models of acute cerebral injury produced a beneficial effect on lesion volume (-1.59, -2.24 to -0.93, $p < 0.001$) and functional outcome (-1.52, -1.88 to -1.15, $p < 0.001$).

Type of HDAC inhibitor

The effects of HDAC inhibition on lesion volume and functional outcome were analysed according to drug type i.e. SB, VPA, TSA or ‘other’ (Fig. 4). A beneficial effect of SB was
seen on both lesion volume (-3.48, -4.93 to -2.04, \( p < 0.001 \)) and functional outcome (-2.01, -2.55 to -1.83, \( p < 0.001 \)). In addition, a beneficial effect of VPA was observed on lesion volume (-2.94, -4.48 to -1.40, \( p = 0.002 \)) and functional outcome (-1.96, -2.62 to -1.29, \( p < 0.001 \)). However, there was no statistically significant beneficial effect of TSA on either lesion volume (\( p = 0.10 \)) or functional outcome (\( p = 0.31 \)). ‘Other’ inhibitors of HDACs included SAHA, ITF2357 and DMA-PB, and beneficial effects were seen on lesion volume (-1.07, -1.52 to -0.61, \( p < 0.001 \)) and functional outcome (-1.36, -1.81 to -0.91, \( p < 0.001 \)).

**Timing of HDAC inhibitor administration in relation to onset of cerebral injury**

In terms of the timing of first administration of the HDAC inhibitor in relation to the onset of cerebral injury, both pre- and post-injury administration were effective at reducing lesion volume and improving functional outcome (Fig. 5). Inhibitors of HDACs were effective at reducing lesion volume when administered -14d to -2h (-2.37, -3.93 to -0.81, \( P = 0.003 \)), -2h to 0h (-3.21, -5.73 to -0.69, \( p = 0.01 \)), 0h to +2h (-1.92, -2.54 to -1.29, \( p < 0.001 \)) or +2h to +14d (-7.75, -11.29 to -4.22, \( p < 0.001 \)). The majority of studies administered the HDAC inhibitor immediately following the onset of cerebral injury (i.e. 0 h to +2 h), with no studies administering a HDAC inhibitor later than +6 h following the onset of cerebral injury in order to measure lesion volume. In terms of assessing functional outcome, a significant effect was reported when the HDAC inhibitor was administered -2 h to 0 h (-1.51, -2.23 to -0.79, \( p < 0.001 \)), 0 h to +2 h (-2.10, -2.66 to -1.53, \( p < 0.001 \)), or +2 h to +14 d (-1.18, -1.68 to -1.22, \( p < 0.001 \)) following onset of cerebral injury. No beneficial effect of the HDAC inhibitor was observed when administered -14 d to -2 h prior to the onset of cerebral injury (\( p = 0.75 \)). When assessing functional outcome, the majority of studies administered the HDAC inhibitor immediately following the onset of cerebral injury (i.e. 0 h to +2 h and +2 h to +14 d), with the latest time of administration being +7 days. Significantly different estimates were found within the timing of administering the HDAC inhibitor for assessing lesion volume (\( \chi^2 = 136.28, df = 28, p < 0.001 \)) and functional outcome (\( \chi^2 = 173.24, df = 44, p < 0.001 \)).

**Discussion**

Inhibitors of HDACs were first considered as a treatment for brain injury when they were observed to block oxidative neuronal death (Ryu et al., 2003); our systematic review of the experimental evidence thus far supports that notion. Although the analysis included fewer published studies than, for example, Gibson et al. (2008) or England et al. (2009), data from significantly greater numbers of individual animals have been assessed, indicating the inclusion of large and complex studies reporting dose-response, timing of administration, and multiple outcome assessments. Functional outcome, in combination with effects on histopathology, may be as important in terms of assessing benefit.

Systematic review does have limitations. Analyses can only include available data, often only accessible in published studies. Negative or neutral studies are less likely to be published and so any result may overstate effect size. Conversely, positive studies involving recently patented compounds might not be available. Non-publication also limits information on the effective dosing regimen and extracting multiple pieces of information from single publications introduces bias. If the positive Egger’s test reported here results from publication bias, this could be explained by the absence of small neutral or negative studies in our analysis.

We assessed quality score of the included studies in accordance with previously published protocols (Macleod et al., 2005; Gibson et al., 2006), and HDAC inhibitors were shown to be effective in those studies with a quality score of 3 and more. It would appear from the publications we analysed that the methodological quality of animal studies can be vastly improved.
improved. For example, few studies stated categorically that experimental subjects were randomized to treatment groups. Randomization prevents systematic errors of sampling and reduces the possibility that treatment efficacy is overestimated. Although sources of variation between experimental animals might be small the literature does support the notion that randomization can influence outcome in experimental animal stroke studies (Crossley et al., 2009; Philip et al., 2009; Sena et al., 2010). Observer blinding was reported only in a fraction of the studies included. Additionally, while most studies reported monitoring physiological parameters, the majority only measure body temperature. Physiologic parameters provide important information regarding treatment and these are critical in the future design of clinical trials. A recent survey highlights the need to more accurately report research using animals (Kilkenny et al., 2009). Useful guidelines have been proposed and, subsequently, adopted by a broad range of scientific journals (Kilkenny et al., 2010).

Inhibitors of the zinc-dependent HDACs are structurally diverse, suggesting different mechanisms of action, and all have some isoform selectivity (Thomas, 2009). The hydroxamates, SAHA and TSA, and the small carboxylates, SB and PB, inhibit Classes I and II. The benzamide, MS-275, and also VPA have a narrower range and target HDACs chiefly in Class I. More specific inhibitors are in development and selective inhibition of HDACs is likely to be very advantageous for the treatment of acute brain injury. Although this review has identified potentially efficacious dose ranges, adequate dose–response relationships were not fully examined. In addition, few studies treated at a time point > 6 h following injury and yet the effectiveness of delayed administration is a very pertinent question with respect to stroke therapy. Our review highlights the fact that no studies assessed the neuroprotective effectiveness of HDAC inhibitors either in females or in ‘aged’ animals. Efficacy of a drug in young, healthy, male animals appears to be a very poor predictor of clinical outcome (see for example, Bath et al., 2010).

Potential explanations for the in vivo neuroprotective effects of HDAC inhibitors are multi-fold (Langley et al., 2009; Marks and Xu, 2009). Following injury to the CNS the rate of neurogenesis increases and HDAC inhibitors can drive endogenous and isolated embryonic and adult neural progenitor cells to differentiate into neurons (Montgomery et al., 2009; Rivieccio et al., 2009). Whether any neuronal re-population ultimately contributes to recovery of function remains moot. The HDACS 1 and 3 associate with HIF-1α and HDAC can modulate transcription via p53. In cultured neurons, HDAC inhibition prevents Bax-mediated apoptosis (Uo et al., 2009). Despite promoting nuclear p53 accumulation, Class I/II HDAC inhibitors such as SB, TSA and SAHA protect postnatal mouse cortical neurons from p53-dependent cell death. The HDAC inhibitors suppress p53-dependent expression of PUMA, a critical signaling intermediate linking p53 to Bax activation, and prevent post-mitochondrial events including cleavage of caspase-9 and caspase-3. Post-injury, convergence of a number of cell death pathways results in changes in mitochondrial morphology and dynamics. Recent findings indicate that HDAC inhibitors preserve the viability of isolated mouse optic nerve (a white matter tract) in the face of oxygen-glucose deprivation, an effect which correlates with preservation of mitochondrial structure/function (Baltan et al., 2010).

In summary, the outcome of this systematic review supports the effectiveness of HDAC inhibitors in preventing and ameliorating acute CNS injury in animals, and underscores those areas where experimental evidence is lacking. The HDACs, with their distinct cellular and sub-cellular localization, and discrete substrates, represent novel and attractive targets in the search for novel therapeutics with which to treat stroke and head injury. A number of the current HDAC inhibitors are already in trial for various cancers and, in the case of SAHA, have been FDA approved.
Acknowledgments

The authors' various research projects are supported by grants from Research into Ageing (CLG), the American Heart Association and NIH/NINDS (SPM).

References

Baltan, S.; Murphy, SP.; Danilov, CA.; Bachleda, A.; Morrison, RS. Program No 345.4. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience; 2010. Class I histone deacetylase inhibition preserves glial cell function and viability to protect white matter from ischemic injury. Online


J Neurochem. Author manuscript; available in PMC 2011 November 1.


J Neurochem. Author manuscript; available in PMC 2011 November 1.


**Abbreviations used**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>confidence interval</td>
</tr>
<tr>
<td>HAT</td>
<td>histone acetyl transferase</td>
</tr>
<tr>
<td>HDAC</td>
<td>histone deacetylase</td>
</tr>
<tr>
<td>SMD</td>
<td>standardized mean difference</td>
</tr>
<tr>
<td>TBI</td>
<td>traumatic brain injury</td>
</tr>
<tr>
<td>WM</td>
<td>white matter</td>
</tr>
</tbody>
</table>
Fig. 1.
The search process and criteria for the exclusion of studies. A total of 12 studies were included. Studies were excluded if they did not report the following: induction of experimental brain injury, administration of HDAC inhibitor, measurement of infarct/functional outcome, or contain primary data. N = number of studies.
Fig. 2.
Funnel plots of precision (reciprocal of SE) against the SMD of all outcome measures for studies utilising models of focal ischemia ($p < 0.001$, A) or other models of acute cerebral injury ($p < 0.001$, B). Asymmetry was formally assessed using the Egger test.
Fig. 3.
Standardized mean difference and 95% CI of all outcome measures by reported study quality score. A beneficial effect of HDAC inhibitor was observed in studies which all had a reported quality score of 2 or greater. N, number of animals; C, number of comparisons; S, number of published studies.
Fig. 4.
Standardized mean difference and 95% CI for different outcome measures (lesion volume and functional outcome) in all studies. Data are grouped according to the type of HDAC inhibitor administered. Both SB and VPA had a significant effect on reducing lesion volume and increasing functional outcome, whereas TSA had no benefit on either outcome. The remaining HDAC inhibitors, grouped as ‘other’, also had a beneficial effect on lesion volume and functional outcome. N, number of animals; C, number of comparisons; S, number of published studies.
Fig. 5.
Standardized mean difference and 95% CI for different outcome measures (lesion volume and functional outcome) in all studies. Data are grouped according to the time of the first administration of drug in relation to the onset of cerebral injury. HDAC inhibitors were beneficial in terms of reducing lesion volume and increasing functional outcome whether administered prior to, or following the onset of cerebral injury. However, no beneficial effect was observed on functional outcome when administered -14d to -2h prior to the onset of cerebral injury. N, number of animals; C, number of comparisons; S, number of published studies.
Table 1

Characteristics of the studies included in the review

<table>
<thead>
<tr>
<th>Study</th>
<th>Parameters assessed</th>
<th>HDAC inhibitor (dose)</th>
<th>First dose timing</th>
<th>Species</th>
<th>Model</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dash et al. 2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>MWM latency (35–42 days)</td>
<td>SB (1.2 g/kg)</td>
<td>+7 days</td>
<td>Male CM</td>
<td>TBI</td>
<td>i.p.</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>MWM latency (14–22 days)</td>
<td>SB (1.2 g/kg)</td>
<td>+0.5 h</td>
<td>Male CM</td>
<td>TBI</td>
<td>i.p.</td>
</tr>
<tr>
<td>Faraco et al. 2006</td>
<td>Infarct volume, mm³ (24 h)</td>
<td>SAHA (12.5, 25, 50, 100 mg/kg)</td>
<td>0 h</td>
<td>Male CM</td>
<td>FCI (P)</td>
<td>i.p.</td>
</tr>
<tr>
<td>Kabakus et al. 2005</td>
<td>Infarct area, mm² (5 days)</td>
<td>VPA (0.2, 0.4 g/kg)</td>
<td>+1 h</td>
<td>Male 7-day WR</td>
<td>NHI</td>
<td>i.p.</td>
</tr>
<tr>
<td>Kim et al. 2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiments 1–2</td>
<td>Infarct volume, mm³ (24 h), NSS (24 h)</td>
<td>VPA (0.3 g/kg)</td>
<td>0 h</td>
<td>Male SDR</td>
<td>FCI (P)</td>
<td>s.c.</td>
</tr>
<tr>
<td>Experiments 3–4</td>
<td>Infarct volume, mm³ (24 h), NSS (24 h)</td>
<td>SB (0.2, 0.3, 0.5, 0.7 g/kg)</td>
<td>0 h</td>
<td>Male SDR</td>
<td>FCI (P)</td>
<td>s.c.</td>
</tr>
<tr>
<td>Experiments 5–6</td>
<td>Infarct volume, mm³ (24 h), NSS (24 h)</td>
<td>TSA (0.5 g/kg)</td>
<td>0 h</td>
<td>Male SDR</td>
<td>FCI (P)</td>
<td>s.c.</td>
</tr>
<tr>
<td>Experiments 7–9</td>
<td>Infarct volume, mm³ (24 h), NSS (24 h), Rotorod (24 h)</td>
<td>VPA (0.3 g/kg)</td>
<td>+3 h</td>
<td>Male SDR</td>
<td>FCI (P)</td>
<td>s.c.</td>
</tr>
<tr>
<td>Experiments 10–15</td>
<td>Infarct volume, mm³ (24 h), NSS (24 h), Rotorod (24 h)</td>
<td>SB (0.3 g/kg)</td>
<td>+3 or +6 h</td>
<td>Male SDR</td>
<td>FCI (P)</td>
<td>s.c.</td>
</tr>
<tr>
<td>Kim et al. 2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiments 1–2</td>
<td>Infarct volume, mm³ (24 h), NSS (24 h)</td>
<td>VPA (0.3 g/kg)</td>
<td>0 h</td>
<td>Male SDR</td>
<td>FCI (P)</td>
<td>s.c.</td>
</tr>
<tr>
<td>Qi et al. 2004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>Infarct volume, % (72 h)</td>
<td>4-PBA (15, 40, 120 mg/kg)</td>
<td>−0.5 h</td>
<td>Male CM</td>
<td>HI</td>
<td>i.p.</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>NSS (1, 2 and 3 days)</td>
<td>4-PBA (40, 120 mg/kg)</td>
<td>0 h</td>
<td>Male CM</td>
<td>HI</td>
<td>i.p.</td>
</tr>
<tr>
<td>Ren et al. 2004</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>Infarct volume, mm³ (24 and 48 h), NSS (24 and 48 h)</td>
<td>VPA (0.3 g/kg)</td>
<td>0 h</td>
<td>Male SDR</td>
<td>FCI (T)</td>
<td>s.c.</td>
</tr>
<tr>
<td>Shein et al. 2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>Infarct volume, mm³ (40 h)</td>
<td>ITF2357 (0.1 g/kg)</td>
<td>+1 h</td>
<td>Male SM</td>
<td>TBI</td>
<td>i.p.</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Neurological score (24 h, 3 days, 6 days, 10 days, 14 days, 21 days)</td>
<td>ITF2357 (0.1 g/kg)</td>
<td>−0.5, +1, +24 h</td>
<td>Male SM</td>
<td>TBI</td>
<td>i.p.</td>
</tr>
<tr>
<td>Sinn et al. 2007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>Infarct volume, % (72 h), LPT (1 d, 28 d), edema volume, % (72 h)</td>
<td>VPA (0.3 g/kg)</td>
<td>+2 h</td>
<td>Male SDR</td>
<td>EIA</td>
<td>i.p.</td>
</tr>
<tr>
<td>Yildrim et al. 2008</td>
<td>Infarct volume, mm³ (24 h), NSS (24 h)</td>
<td>TSA (5 mg/kg)</td>
<td>−14 days</td>
<td>Male SVM</td>
<td>FCI (T)</td>
<td>i.p.</td>
</tr>
<tr>
<td>Zhang et al. 2008</td>
<td>% DN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MWM, Morris water maze; NSS, neurological severity scale; LPT, limb placement test; %DN, % degenerating neurones; SB, sodium butyrate; SAHA, suberoylanilide hydroxamic acid; ITF2357, a hydroxamic acid moiety linked to an aromatic ring, synthesized at Italfarmaco; VPA, valproic acid; TSA, trichostatin A; 4-PBA, sodium 4-phenylbutyrate; DMA, 4-dimethylamino-N-[5-(2-mercaptoacetyl)amino]pentyl]benzamide; CM, C57 mice; WR, Wistar rats; SDR, Sprague–Dawley rats; SM, Sabra mice; SVM, 129/SV wild-type mice; TBI, traumatic brain injury; FCI (P), focal cerebral
ischemia (permanent); NHI, neonatal hypoxic ischemia; HI, hypoxia–ischemia; FCI (T), focal cerebral ischemia (transient); EIA, experimental intracerebral hemorrhage; i.p., intra-peritoneal; s.c., subcutaneous.