ORIGINAL INVESTIGATION

HDAC6-selective inhibitors decrease nerve-injury and inflammation-associated mechanical hypersensitivity in mice

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Abstract

Background HDAC6 is a class IIB histone deacetylase expressed at many levels of the nociceptive pathway. This study tested the ability of novel and selective HDAC6 inhibitors to alleviate sensory hypersensitivity behaviors in mouse models of peripheral nerve injury and peripheral inflammation.

Methods We utilized the murine spared nerve injury (SNI) model for peripheral nerve injury and the Complete Freund's Adjuvant (CFA) model of peripheral inflammation. We applied the Von Frey assay to monitor mechanical allodynia.

Results Using the SNI model, we demonstrate that daily administration of the brain-penetrant HDAC6 inhibitor, ACY-738, abolishes mechanical allodynia in male and in female mice. Importantly, there is no tolerance to the antiallodynic actions of these compounds as they produce a consistent increase in Von Frey thresholds for several weeks. We observed a similar antiallodynic effect when utilizing the HDAC6 inhibitor, ACY-257, which shows limited brain expression when administered systemically. We also demonstrate that ACY-738 and ACY-257 attenuate mechanical allodynia in the CFA model of peripheral inflammation.

Conclusions Overall, our findings suggest that inhibition of HDAC6 provides a promising therapeutic avenue for the alleviation of mechanical allodynia associated with peripheral nerve injury and peripheral inflammation.

Keywords Neuropathic pain · Histone deacetylase · ACY-738 · ACY-257 · Peripheral inflammation · Peripheral nerve injury

Introduction

Chronic pain is characterized by a range of sensory abnormalities, cognitive deficits, and mood disorders (Cruccu 2007; Mitsi and Zachariou 2016). Chronic pain conditions represent a major therapeutic challenge as most of the available treatments show poor efficacy, alleviating only a subset of sensory signs while producing major side effects (Ling et al. 2011;

Reisner 2003; Schloesser et al. 2012; Sultan et al. 2008). A better understanding of the cellular and molecular mechanisms that modulate the transmission and maintenance of pain is necessary for the development of novel therapeutic treatments.

Several recent studies have provided a proof of concept that the pharmacological modulation of histone deacetylases (HDACs) may serve as a possible avenue for the development of new treatments. Prolonged pain-like states alter the composition and function of HDAC-containing multiprotein complexes, which affect chromatin states and other processes impacting gene expression associated with sensory hypersensitivity and anxiodepressive behaviors (Bai et al. 2010; Denk et al. 2013; Descalzi et al. 2015; Matsushita et al. 2013). Indeed, broad inhibition of HDACs using hydroxamate HDAC inhibitors, the most clinically advanced chemical agents targeting chromatin, ameliorates several signs of peripheral nerve injury in preclinical studies (Denk et al. 2013; Matsushita et al. 2013; Zammataro et al. 2014). Furthermore, broadly acting HDAC inhibitors, such as trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), relieve CFA-

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induced (Mao et al. 2019) or nerve injury-induced (Matsushita et al. 2013) thermal hyperalgesia after only a few days of administration. This rapid action contrasts with that of current first-line therapeutics, such as monoamine targeting antidepressants, that require weeks of treatment (Magni 1991; Reisner 2003; Ziegler and Fonseca 2015). HDAC6 and HDAC10 form class IIB HDACs, which reside exclusively in the cytoplasm unlike class I or class IIA HDACs that permanently or partially reside in the nucleus. Class IIB HDACs have a tissue-specific distribution, and they do not intrinsically deacetylate histones in vivo (Falkenberg and Johnstone 2014; Morris and Monteggia 2013). HDAC6 has been shown to be devoid of deacetylase activity on histones in vivo but, instead, functions as a deacetylase for alpha tubulin, cortactin, and heat shock protein 90 (HSP90); it thereby modulates microtubule formation and the trafficking of nuclear receptors and growth factors such as brain-derived neurotrophic factor (BDNF) (Haggarty et al. 2003; Hubbert et al. 2002; Tao et al. 2015). Earlier studies have shown that HDAC6 loss of function in the nucleus raphe as well as HDAC6 pharmacological inhibition promote resilience to chronic stress and potentiate antidepressant responses in murine models of depression (Fukada et al. 2012; Jochems et al. 2014, 2015). More recent preclinical studies have demonstrated that selective HDAC6 inhibitors alleviate behavioral manifestations of chemotherapy-induced peripheral nerve injury (Benoy et al. 2017; Krukowski et al. 2017; Van Helleputte et al. 2018).

The development and maintenance of central sensitization in response to nerve injury or inflammation involve both peripheral and central mechanisms (Ossipov et al. 2000; Suzuki and Dickenson 2005). Based on evidence that HDAC6 inhibitors exert their actions in peripheral and central sites (Benoy et al. 2017; Jochems et al. 2014; Krukowski et al. 2017; Ma et al. 2019; Van Helleputte et al. 2018), we tested the ability of two selective HDAC6 inhibitors to alleviate sensory hypersensitivity behaviors. In this study, we applied murine models of peripheral nerve injury and hind paw inflammation to test the antiallodynic properties of the blood-brain barrier–permeable compound ACY-738 (Jochems et al. 2014; Jones and Jarpe 2013) and the peripherally restricted compound ACY-257 in models of long-term nerve injury and hind paw inflammation. Our findings from male and female C57BL/6 J mice suggest that treatment with ACY-738 alleviates mechanical allodynia in the spared nerve injury (SNI) model of peripheral nerve injury and in the Complete Freund's Adjuvant (CFA) model of peripheral inflammation. The peripherally restricted ACY-257 also alleviates mechanical allodynia in the SNI and CFA models, suggesting that inhibition of HDAC6 in the periphery may be used for alleviation of sensory hypersensitivity signs. Overall, our findings suggest that inhibition of HDAC6 provides a novel and efficacious way to alleviate mechanical allodynia associated with peripheral nerve injury or hind paw inflammation.

Methods

Subjects

For mouse behavioral experiments performed at Icahn School of Medicine at Mount Sinai, New York, USA, we used 2- to 3-month-old male and female C57BL/6 J mice (Jackson laboratories, Bar Harbor, USA). Mice were housed with a 12-h dark/light cycle according to the Institutional Animal Care and Use Committee (IACUC) of the Icahn School of Medicine at Mount Sinai.

Male C57BL/6J mice from SLAC Laboratory Animal Co. Ltd. were used for pharmacokinetic studies performed at Shanghai ChemPartner Co., Ltd. Mice were group housed (5 per cage) on a 12-h light/dark cycle, provided with sterile food and water ad libitum. All procedures have been approved by AAALAC, and the studies were conducted in accordance with IACUC-approved protocols of Shanghai ChemPartner Co., Ltd.

Sprague-Dawley rats, (Harlan Israel) 120–140 g at study initiation, were used for tail flick studies performed at MD Biosciences Inc., Ness Ziona, Israel. Animals were housed within a limited access rodent facility and kept in groups with a maximum of 3 rats per polypropylene cage. The cages were fitted with solid bottoms and filled with sterile wood shavings as bedding material and provided with sterile food and water ad libitum. Environmental conditions are set to maintain temperature at 20–24 °C, RH 30–70%, and 15–30 air changes/h. All procedures and experiments were approved by the M.D. Biosciences IACUC committee with adherence to National Institutes of Health guidelines.

Drugs

Compounds were provided by Regenacy Pharmaceuticals, Boston, USA. Compounds were administered through food pellets, p.o. dosing, or i.p. injections. Custom made sterilizable rodent diet (Envigo, Indianapolis, USA) containing 100 mg/kg ACY-738 was provided ad libitum. Pellets were added in the morning of treatment day 1 and removed after the last treatment day immediately following behavioral assessment. Given that mice consume about 10% of their body weight, the estimated pellet dose is 10–15 mg/kg per day. Control mice were given regular chow (food pellets). For the rat p.o. dosing, ACY-738 was dissolved in 0.5% hydroxypropyl methylcellulose in distilled water. The drugs were dissolved at a concentration to ensure the dosing volume was 10 ml/kg. For i.p. injections in rat studies, ACY-738 was suspended in 10% DMSO and 90% of 5% dextrose in water. ACY-257 was suspended in 10% DMSO and 90% of 5% dextrose solution (for experiment in Fig. 4b) or dissolved in 30% DMSO, 40% PEG 300, and 30% of 5% dextrose solution (for experiments in Fig. 4a and c). For mouse behavioral

studies, drugs were injected at a volume of 100 μl, and Von Frey measurements were taken 1 h after drug treatment.

HDAC enzymatic assays

Potency of the compound was determined using recombinant enzymes and a kinetic method described previously (Bradner et al. 2010). N-terminal GST-tagged HDAC enzymes (BPS Biosciences, San Diego, USA) expressed in a baculoviral system and purified by affinity chromatography and gel filtration were diluted to 1.5-fold final concentration in assay buffer and pre-incubated with test compounds for 10 min before the addition of substrate, FTS (HDAC1, HDAC2, HDAC3, and HDAC6). The amount of substrate for each enzyme was equal to the Michaelis constant (Km), as determined by a titration curve. The enzymatic reaction was monitored over 30 min for release of 7-amino-4-methoxy-coumarin after deacetylation of the lysine side chain in the peptide substrate, and the linear rate of the reaction was calculated (Jochems et al. 2014).

Pharmacokinetic profiling

Studies were performed by Shanghai ChemPartner Co., Ltd (Shanghai, China). Male C57Bl/6J mice were dosed with 5 mg/kg i.p. ACY-257. Blood samples were collected by retro-orbital puncture at 5, 15, and 30 min and 1, 2, 4, 8, and 24 h. At 5 and 15 min and at 1 and 4 h following administration, brains were collected, rinsed with saline, and snap frozen. Plasma and brain homogenates were extracted with acetonitrile, and the level of test article was measured by LC/MS/MS and quantified against a standard curve in either plasma or brain homogenate, as appropriate. Maximum plasma or brain concentration (Cmax) and the area under the curve (AUC) were calculated using WinNonLin software.

SNI operation

SNI was performed on the left sciatic nerve, as previously described (Descalzi et al. 2017; Mitsi et al. 2015; Shields et al. 2003). Briefly, a skin and muscle incision of the left hind limb at mid-thigh level was performed using a stereomicroscope. The common peroneal and the sural nerves were carefully ligated with 6.0 silk sutures (Johnson and Johnson, New Brunswick, USA and Patterson Veterinary, Devens, USA) distally transected, and 1-2 mm sections of these nerves were removed, while the tibial nerve was left intact. Skin and muscle were then closed with silk 4.0 sutures (Johnson and Johnson, New Brunswick, USA and Patterson Veterinary, Devens, USA). In the sham-operated group, skin and muscle separation was performed, but the nerves were left intact. Mice were left to recover from surgery for at least 1 week before taking SNI baseline measurements in the Von Frey assay.

CFA treatment

Left hind paw inflammation was induced by intraplantar injection of 25-μl Complete Freund's Adjuvant ((CFA), Sigma-Aldrich, St. Louis, USA), diluted 1:1 in saline as previously described (Gaspari et al. 2018). CFA BL measurements were taken 18–24 h after CFA treatment.

Von Frey test for mechanical allodynia

For the monitoring of mechanical allodynia, we used the Von Frey paradigm with ascending forces expressed in grams (Stoelting Co, Wood Dale, USA) (Descalzi et al. 2017; Mitsi et al. 2015). Each filament was applied five times in a row against the central area of the ipsilateral paw. Hind paw withdrawal or licking was marked as a positive response. The threshold was defined as the second filament size at which at least three out of five applications elicit a nocifensive response.

Hot plate assay

Mice were habituated in the room for 2 h, and nociceptive latencies were measured using a 52 °C hot plate apparatus (IITC Life Sciences, Woodland Hills, USA). A cutoff of 40 s was established to avoid inflammation or tissue damage (Gaspari et al. 2018). After baseline measurement, pellets were provided and test measurement was performed 3 days after pellet treatment. A positive response includes jumping, licking, or flinching of either paw.

Tail flick assay

The tail flick study (Ugo Basile Tail Flick instrument, Trappe, USA) was performed at MD Biosciences Inc., Ness Ziona, Israel. Male Sprague-Dawley rats were tested for baseline reaction to a constant intensity (50 units)-infrared light source on their tails (D'Amour and Smith 1941). Flicking the tail out of the path of the light source indicated a nocifensive reaction, and the latency time to tail flick was measured. Three days following the baseline measurement, rats were dosed with drug 5 mg/kg s.c. morphine, 10 mg/kg ACY-738 (by oral gavage), or respective vehicles. The latency time of the reaction to the infrared light source was tested at 1, 2, 3, and 4 h post-dosing by an experimenter blind to the dosing groups.

Statistical analysis

For the experiments (Figs. 1, 2, 3a, 4) monitoring behavior of the same group of mice over time, we used two-way repeated measures ANOVAs followed by Holm-Sidak's post hoc tests.
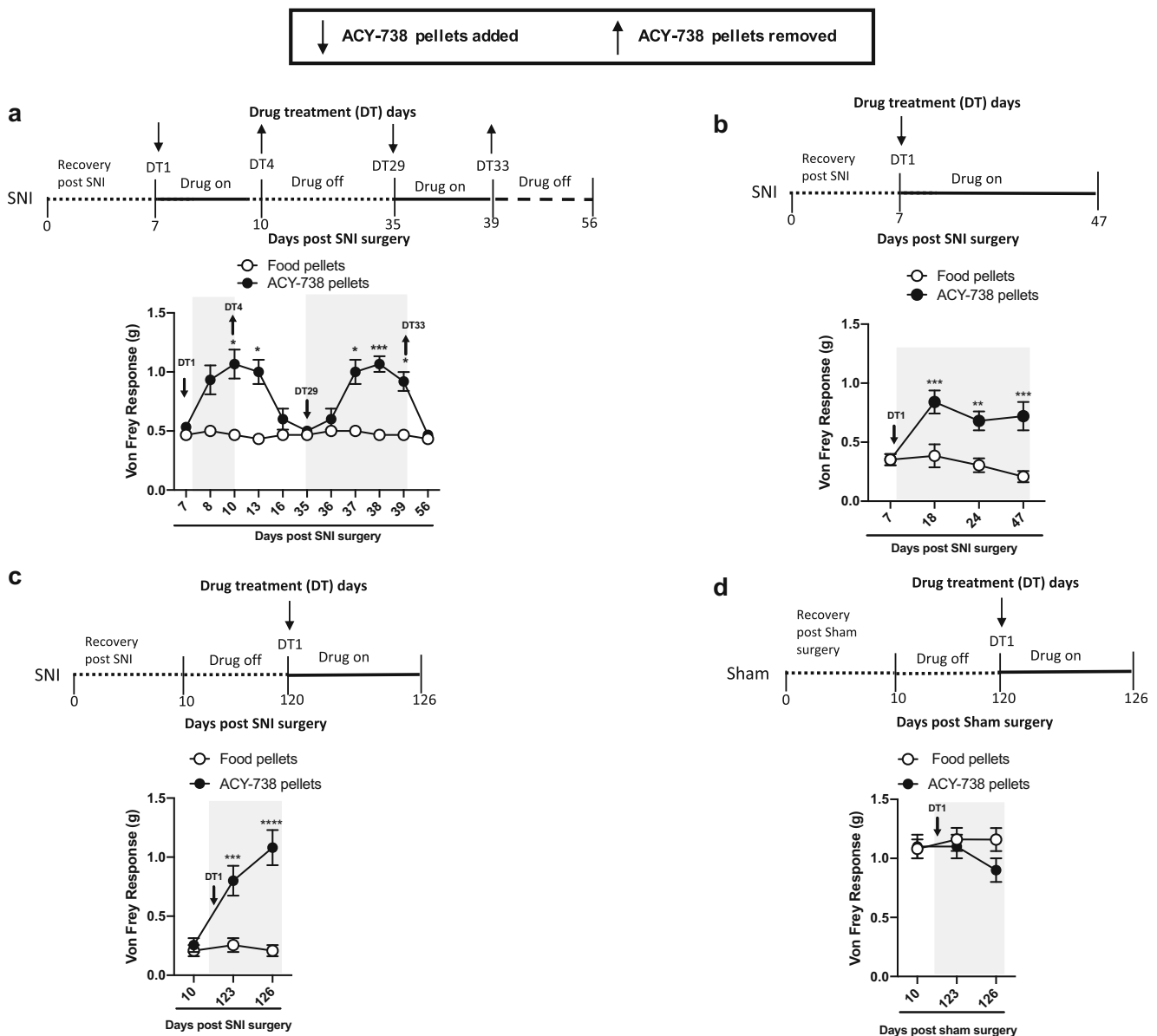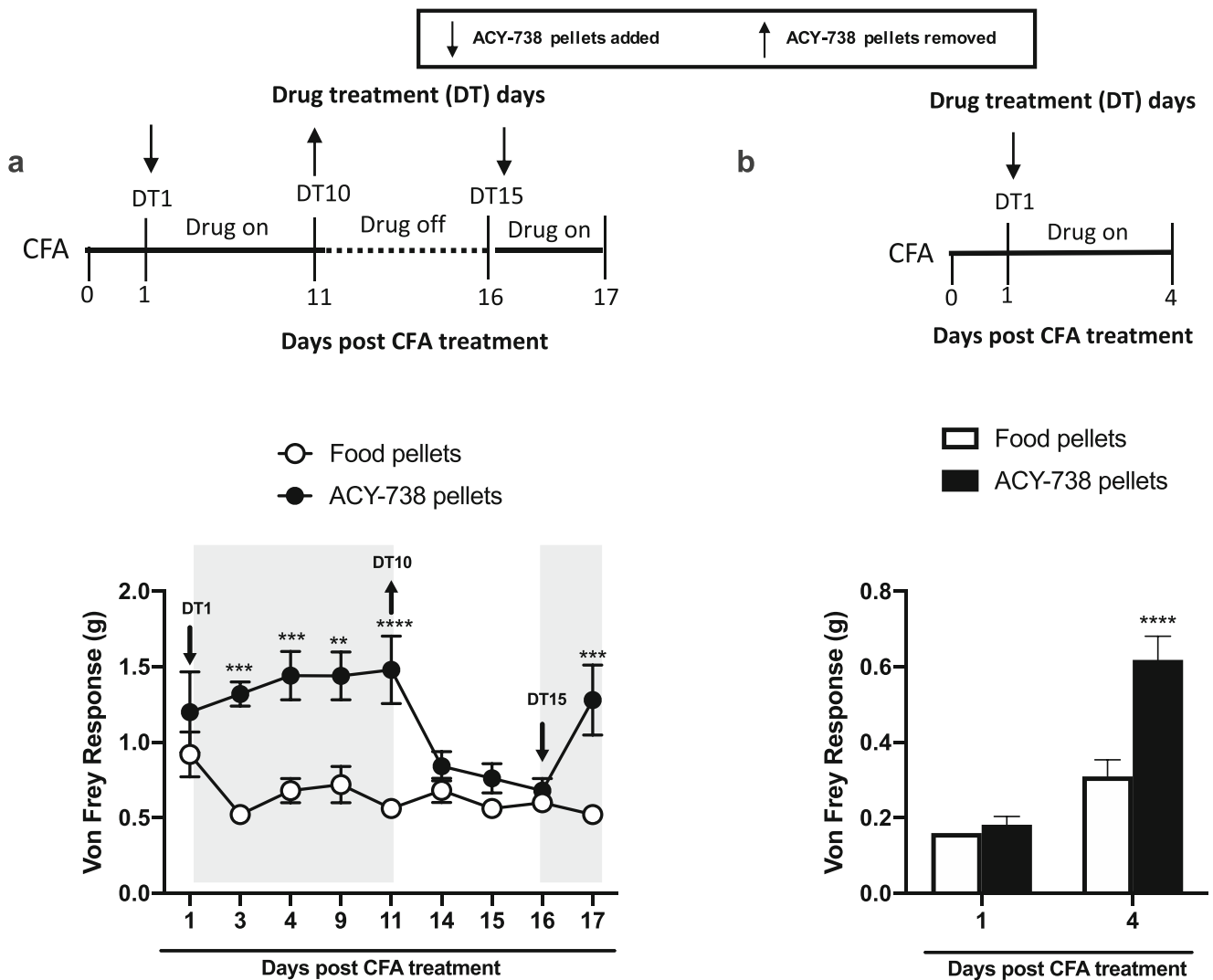
Fig. 1 ACY-738 alleviates mechanical allodynia in the SNI model of peripheral nerve injury. Down arrows indicate when ACY-738 pellets were added, and up arrows indicate when ACY-738 pellets were removed. Shaded areas on figures indicate the duration of drug treatment. A significant interaction between day of measurement x treatment was followed by a Holm-Sidak's post hoc test. **a** Mice treated with ACY-738 pellets show reduced mechanical allodynia in the Von Frey assay, compared with control mice treated with regular food pellets. Numbers on arrows indicate the day of drug treatment (DT) relative to drug treatment initiation (DT1). Drug treatment started immediately after SNI baseline (7 days after surgery) measurement ($n = 6$, two-way ANOVA followed by Holm-Sidak's post hoc test, $F(10,99) = 8.57$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). **b** Long-term treatment with ACY-738 does not lead to tolerance, as mice show the same antiallodynic response throughout the

47 days of treatment. Number on down arrow indicates the day of drug treatment (DT) initiation. Drug treatment started the same day after SNI baseline measurement (7 days after surgery, $n = 5$, two-way ANOVA followed by Holm-Sidak's post hoc test, $F(3,24) = 4.13$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). **c** Mice under long-term SNI (4 months) show significant reduction of mechanical allodynia when treated with ACY-738 pellets. Control animals received regular food pellets. Number on down arrow indicates the day of drug treatment (DT) initiation. Drug administration started 4 months (day 120) after the induction of SNI ($n = 5$, two-way ANOVA followed by Holm-Sidak's post hoc test, $F(2,14) = 13.31$, $***P < 0.001$, $****P < 0.01$). **d** ACY-738 pellets have no effect on sham-operated mice ($n = 5$), two-way ANOVA. Sham surgeries were performed at the same time as SNI. Number on down arrow indicates the day of drug treatment (DT) initiation

A one-way ordinary ANOVA was used to monitor the effect of drugs over different time periods (Figs. 3b, c, and d) followed by Holm-Sidak's post hoc tests. Error bars are depicting ± SEM. F values for each data set are provided in the figure legends.

Results

Earlier work has demonstrated the selectivity of ACY-738 for HDAC6 (Jochems et al. 2014). ACY-257 also exhibits HDAC6

Fig. 2 Treatment with ACY-738 pellets significantly alleviates mechanical allodynia in the CFA model of peripheral inflammation. Numbers on arrows indicate the day of drug treatment (DT) relative to drug treatment initiation (DT1). Shaded areas on figures indicate the duration of drug treatment. A significant interaction between day of measurement x treatment was followed by a Holm-Sidak's post hoc test. **a** ACY-738 pellet treatment promotes antiallodynic effects in male mice injected with CFA on the left hind paw. Dru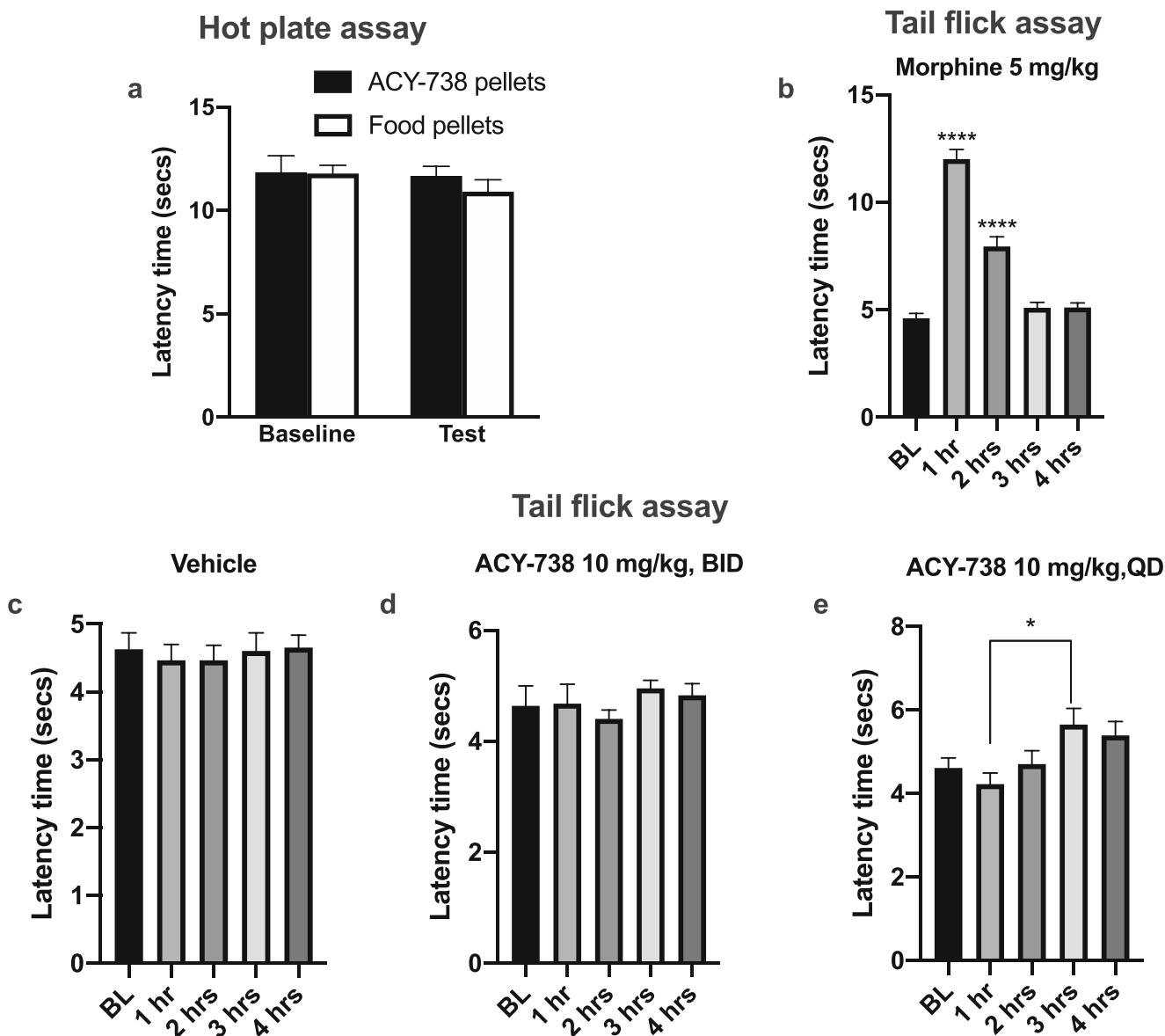g treatment started immediately after CFA-baseline measurement ($(n = 5)$, two-way ANOVA followed by Holm-Sidak's post hoc test, $F_{(8,64)} = 3.80$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). **b** ACY-738 pellet treatment promotes antiallodynic effects in female mice treated with CFA injection on the left hind paw. Drug treatment started immediately after CFA baseline measurement $((n = 8–11)$, two-way ANOVA followed by Holm-Sidak's post hoc test, $F_{(1,17)} = 10$, $**P < 0.0001$)

selectivity, as shown by substrate deacetylation assays in Table 1. ACY-738 and ACY-257 show similar activity but different potencies (1.7 nM and 12 nM, respectively) for the inhibition of HDAC6. While both compounds contain a hydroxamic acid zinc binding group, they differ in structure and in pharmacokinetic properties. ACY-738 is brain-penetrant, with a ratio of brain-to-plasma exposure of 1.22:1 (Jochems et al. 2014), while systemically administered ACY-257 (5 mg/kg, i.p.) is not detected at a biologically active concentration when measured in the mouse brain area under the curve (AUC); brain/AUC plasma ratio, 0.01; $n = 3$ per group, (Table 1).

We hypothesized that pharmacological inhibition of HDAC6 facilitates recovery from sensory hypersensitivity,

and we applied the selective HDAC6 inhibitors ACY-738 and ACY-257 to test this hypothesis. We used the murine SNI paradigm of peripheral nerve injury (Descalzi et al. 2017; Mitsi et al. 2015; Shields et al. 2003) to determine if treatment with ACY-738 alleviates mechanical allodynia. We used the Von Frey assay to monitor mechanical allodynia responses of adult groups of male C57BL/6 J mice at different time points after unilateral (left side) SNI surgery (Descalzi et al. 2017; Mitsi et al. 2015; Shields et al. 2003). As shown in Fig. 1, mice treated with ACY-738 food pellets show increased mechanical thresholds compared with controls (regular food pellets). ACY-738 pellets were provided to the mice immediately after SNI baseline measurement. A significant

Hot plate assay

Tail flick assay
Morphine 5 mg/kg

Tail flick assay

Vehicle

ACY-738 10 mg/kg, BID

ACY-738 10 mg/kg, QD

Fig. 3 ACY-738 does not alleviate nociceptive responses in the tail flick assay and hot plate assay. **a** No antinociceptive effect was observed in the hot plate assay in mice treated with ACY-738 pellets for 3 days, ($n = 8$), two-way ANOVA. **b** Rats treated with morphine (5 mg/kg s.c.) show an antinociceptive response in the first 2 hours of testing (($n = 8$), one-way ANOVA, followed by Holm-Sidak's post hoc test, $F(4,35) = 0.93$, ****$P < 0.0001$). **c** No difference in tail flick latency was observed in vehicle treated rats ($n = 8$), one-way ANOVA. **d** No antinociceptive effect was observed in the tail flick assay in groups treated with ACY-738 (10 mg/kg p.o.) twice daily (BID) ($n = 8$), one-way ANOVA. **e** No antinociceptive effect was observed with ACY-738 (10 mg/kg p.o.) once a day (QD) except for a small difference in tail flick latency between 1 and 3 h of testing ($n = 8$), one-way ANOVA

antiallodynic response was observed within 3 days of pellet treatment (day10 after SNI surgery on Fig. 1a). While ACY-738 has a quick onset of action, the antiallodynic response is not maintained upon discontinuation of drug treatment (Fig. 1a). Importantly, there is no tolerance to the antiallodynic effects of ACY-738 as daily treatment with the drug increases Von Frey thresholds throughout the 47-day monitoring period (Fig. 1b).

To determine if ACY-738 alleviates mechanical allodynia when treatment starts several months after the induction of peripheral nerve injury, we administered ACY-738 food pellets beginning 4 months after the induction of SNI. Consistent with our earlier findings, treatment of adult male C57BL/6 J mice with ACY-738 significantly elevated Von Frey thresholds relative to that of controls (regular food pellets) (Fig. 1c–d). Treatment with ACY738 or vehicle (regular food) pellets began on day 120 after SNI or Sham treatment, and Von Frey responses were monitored on days 123 and 126.
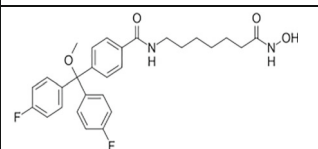
Next, we used the CFA paradigm in male C57BL/6 J mice to determine if ACY-738 alleviates mechanical allodynia
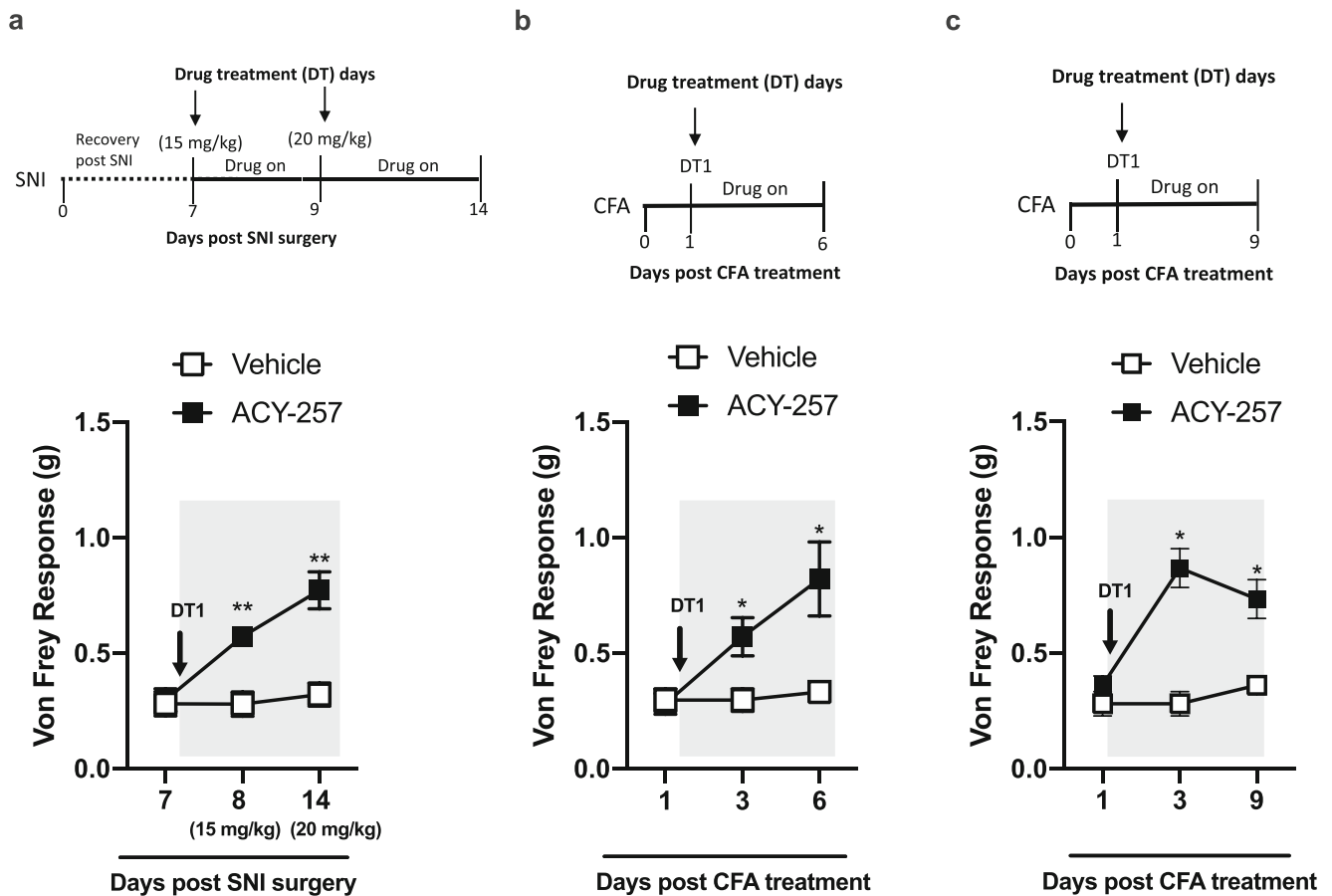
Fig. 4 ACY-257 alleviates mechanical allodynia in the SNI model of peripheral nerve injury and in the CFA model of peripheral inflammation. Numbers on down arrows indicate the day of drug treatment (DT) initiation. Shaded areas on figures indicate the duration of drug treatment. A significant interaction between day of measurement x treatment was followed by a Holm-Sidak's post hoc test. **a** SNI group of male C57BL/6 J mice treated with ACY-257 (15 mg/kg i.p. for 2 days and 20 mg/kg i.p. for a week) shows reduction of mechanical allodynia in the Von Frey assay. Drug or vehicle treatment began immediately after SNI baseline measurement ($n = 6$–7, two-way ANOVA followed by Holm-Sidak's post hoc test, $F_{(2,33)} = 7.83$, $*P < 0.05$). **b** CFA group of male mice treated with ACY-257 (20 mg/kg i.p.) show recovery from mechanical allodynia. Treatment started immediately after baseline measurement ($n = 7$–8, two-way ANOVA followed by Holm-Sidak's post hoc test, $F_{(2,26)} = 4.99$, $*P < 0.05$). **c** Similarly, the CFA group of female mice treated with ACY-257 (20 mg/kg i.p.) recover from mechanical allodynia. Drug treatment started immediately after CFA baseline measurement (($n = 6$), two-way ANOVA followed by Holm-Sidak's post hoc test, $F_{(2,20)} = 7.49$, $*P < 0.05$)

Table 1 Pharmacology and pharmacokinetics of ACY-257

ACY-257 Structure	HDAC1 IC_{50} nM	HDAC2 IC_{50} nM	HDAC3 IC_{50} nM	HDAC6 IC_{50} nM	Cmax ng/ml	T1/2 (h)	Plasma AUC_{last} (hr*ng/mL)	AUC Brain/AUC Plasma Ratio
	268	530	850	12	1047	8.15	871 (5 mg/kg i.p.)	0.01

associated with long-term hind paw inflammation (Avrampou et al. 2019). Adult male mice were injected in the left hind paw with CFA, and Von Frey responses were monitored over a period of 3 weeks. Similar to our observations in the SNI model, ACY-738 promotes a significant antiallodynic response (Fig. 2a) within 2 days of treatment. ACY-738 pellets were provided to the mice immediately after CFA baseline measurement, and a significant antiallodynic response was observed within 2 days of pellet treatment. This response was not maintained when the drug had been discontinued after 10 days. Notably, ACY-738 promotes similar antiallodynic effects in a CFA group of female C57BL/6 J mice (Fig. 2b).

In the next set of studies, we tested whether ACY-738 alleviates responses to acute noxious heat (52 °C) in mice, using a hot-plate procedure. First, male mice were treated with ACY-738 pellets for 3 days, in order to determine if this treatment regimen alters nociceptive thresholds in the hotplate assay. As shown in Fig. 3a, ACY-738 does not have any thermal antinociceptive effect, as hot plate latencies 3 days after drug treatment are similar to those observed at baseline. Next, we applied the tail flick assay, and we monitored tail withdrawal latencies in male Sprague-Dawley rats treated with morphine, ACY-738, or their respective vehicle controls. As shown in Fig. 3c–e, while morphine promotes a significant thermal antinociceptive response in the tail flick assay, treatment with ACY-738 does not affect tail withdrawal latencies. These data support the hypothesis that ACY-738 modulates sensory hypersensitivity, but it does not impact acute thermal nociceptive thresholds.

The next set of experiments investigated if treatment with the peripherally acting compound ACY-257 attenuates SNI-induced mechanical allodynia in the Von Frey assay. Male C57BL/6 J mice were injected with 15 mg/kg i.p. for 2 days, and this treatment significantly increased Von Frey thresholds (Fig. 4a). We continued treatment with 20 mg/kg i.p. daily for 1 week, which produced the same response as the 15 mg/kg regimen. (Fig. 4a). A significant antiallodynic effect was also observed in the CFA model of peripheral hind paw inflammation. Figure 4b and c demonstrates that treatment with ACY-257 (20 mg/kg, i.p.) significantly alleviates mechanical allodynia in male and female groups of C57BL/6 J mice.

Discussion

Our findings demonstrate that the HDAC6 inhibitors ACY-738 and ACY-257 have effective antiallodynic effects in models of peripheral nerve injury and peripheral inflammation. The antiallodynic actions of ACY-738 and ACY-257 were observed in both male and female mice. Interestingly, ACY-257 mediates antiallodynic effects primarily through peripheral mechanisms as this compound does not cross the blood-brain barrier.

The antiallodynic effect of ACY-738 and ACY-257 in SNI-induced mechanical allodynia C57BL/6 J mice is comparable to the effect we observed in earlier studies using the tricyclic antidepressants desipramine (DMI) or the selective serotonin/norepinephrine inhibitor (SNRI) duloxetine (Descalzi et al. 2017; Mitsi et al. 2015). While it takes several days of treatment for antidepressants to promote antiallodynic effects in models of sciatic nerve injury (Alba-Delgado et al. 2018; Bohren et al. 2010; Choucair-Jaafar et al. 2014; Megat et al. 2015; Yalcin et al. 2009), administration of the HDAC6 inhibitors, ACY-738 and ACY-257, leads to significant alleviation of mechanical allodynia within 2–3 days of treatment.

Morphine and other opioid analgesics also have antiallodynic properties, but their long-term use promotes mechanical and thermal hyperalgesia, as well as analgesic tolerance and often physical dependence (Gaspari et al. 2018; Jamison and Mao 2015; Matthes et al. 1996; Webster 2017). Here we report that there is no tolerance to the antiallodynic actions of HDAC6 inhibitors as ACY-738 promotes a consistent antiallodynic response when administered for several weeks (Fig. 1). As mentioned, misuse of opioid analgesics may lead to physical dependence or addiction (Volkow et al. 2019). To date, there is no evidence on a role of HDAC6 in addiction vulnerability; we expect that the antiallodynic and antidepressant actions of HDAC6 involve peripheral sites or midbrain circuits and HDAC6 inhibitors therefore do not contain the risk of addiction. However, it will be important to address whether HDAC6 inhibitors possess rewarding or reinforcing properties and if they alleviate sensory hypersensitivity in opioid-dependent subjects.

Notably, our findings suggest that daily injections or food pellets of ACY-738 or ACY-257 are required for the maintenance of the antiallodynic response, and Von Frey thresholds return to pre-treatment levels within 2 days after the cessation of treatment (Fig. 1). In earlier studies, Krukowski and colleagues revealed that 2-week treatment with the HDAC6 inhibitor ACY-1083 is sufficient to promote recovery from sensory hypersensitivity in a model of chemotherapy-induced neuropathy (CIPN). Furthermore, recovery from sensory hypersensitivity was observed up to 1 month after the end of ACY-1083 treatment (Krukowski et al. 2017). These studies also report that antiallodynic responses were maintained up to 4 days after the last administration ACY-1083 when mice were treated with the drug for only 1 week. Similar observations were made when compound ACY-1215 had been administered for 2 weeks: antiallodynic responses were maintained up to 1 week after drug cessation (Krukowski et al. 2017). In the current study, we monitored Von Frey responses after the end of ACY-738 treatment in experiments of Fig. 1a, whereas the drug was consecutively administered for less than 2 weeks. Future studies will determine if four or more weeks of daily treatment with compounds ACY-738 or ACY-257 (or with other selective HDAC6 inhibitors) are sufficient to

permanently reverse mechanical allodynia in the SNI model. Importantly, the murine SNI model involves truncation of two branches of the sciatic nerve, while the model of cisplatin-induced nerve injury model promotes milder, dispersed peripheral nerve injury which can be reversed by pharmacological treatments. We speculate that the observed antiallodynic effects of HDAC6 inhibitors in our study at least partially involve increased alpha-tubulin acetylation in the dorsal root ganglia neurons as well as actions at other levels of the nociceptive pathway.

HDAC inhibitors have clinical utility in treating several central nervous system diseases such as Alzheimer's, Parkinson's, and multiple sclerosis (Seidel et al. 2015). Interestingly, HDAC6 inhibitors have also been reported to act as antidepressants in rodent models and to potentiate the antidepressant activity of monoamine reuptake inhibitors (Fukada et al. 2012; Jochems et al. 2014). Recent studies have characterized the pharmacodynamics, pharmacokinetics, and antidepressant properties of the novel HDAC6-selective inhibitor, ACY-738; they reported that administration of ACY-738 to HDAC6 KO mice has no response in in vivo assays and shows no increase in acetylated tubulin levels (Jochems et al. 2014). Here we demonstrate that ACY-257 shows high selectivity for HDAC6. We expect that, at the doses used, ACY-738 and ACY-257 selectively inhibit HDAC6, and future studies will further evaluate potential off-site effects.

Our group and others have demonstrated that prolonged peripheral nerve injury in mice promotes anxiety-like behaviors, loss of motivation, vulnerability to stress, and the development of anhedonia-like behaviors. Treatment with DMI or SNRIs alleviates depression-like behaviors in models of peripheral nerve injury (Descalzi et al. 2017; Kremer et al. 2016; Mitsi et al. 2015). HDAC6 inhibitors have also demonstrated in vivo efficacy to reverse chemotherapy-induced cognitive impairment (Ma et al. 2018). Given the high comorbidity between chronic neuropathic pain and major depression, future work will determine if selective HDAC6 inhibitors alleviate anxiety- and depression-like behaviors associated with prolonged nerve injury. While the actions of monoamine targeting antidepressants involve both midbrain structures and the brain reward center (Russo and Nestler 2013), HDAC6 inhibitors may have minimal effects on the brain reward pathway as HDAC6 is primarily expressed in serotonin circuits of the midbrain (Jochems et al. 2014). However, it is likely that HDAC6 is present in the axons of neurons projecting to the nucleus accumbens (NAc) or to the ventral tegmental area (VTA); as a result, HDAC6 inhibitors may indirectly control synaptic activity in the brain reward center.

While the HDAC6 inhibitors ACY-738, ACY-257, ACY-1083, and ACY-1215 potently alleviate sensory hypersensitivity behaviors in models of peripheral nerve injury and peripheral inflammation, the detailed mechanism of HDAC6 action remains unknown. As mentioned, the highest expression levels of HDAC6 mRNA and protein are observed in brain structures mediating nociceptive responses such as the nucleus raphe, the periaqueductal gray, the dorsal root ganglia, and peripheral nerve endings. The peripheral actions of HDAC6 inhibitors are documented by studies using the cisplatin model of chemotherapy-induced neuropathy. These studies show that treatment with ACY-1083 normalizes mitochondrial function in the dorsal root ganglia and in the tibial nerve and restores intraepidermal innervation. Similar effects were reported by Ma et al. (Ma et al. 2019) with ACY-1215. Our findings from experiments using compound ACY-257 suggest that inhibition of HDAC6 action in the periphery is sufficient to attenuate mechanical allodynia (Fig. 4). Further evaluation of peripherally acting HDAC6 inhibitors in models of nerve injury and inflammation will determine if such compounds successfully alleviate sensory hypersensitivity behaviors beyond allodynia. Peripherally acting HDAC6 inhibitors may provide an efficient avenue for pain management as they are expected to be devoid of CNS side effects. On the other hand, it is important to further characterize centrally acting HDAC6 inhibitors and to determine whether HDAC6 actions in the brain alleviate affective signs of nerve injury and anxiodepressive states.

Overall, our findings suggest that selective HDAC6 inhibitors possess strong antiallodynic properties in murine models of peripheral nerve injury and peripheral inflammation, pointing to novel pharmacological avenues for the management of pathological pain symptoms.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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