HDAC inhibitor attenuates rat traumatic brain injury induced neurological impairments

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ABSTRACT

Oxidative stress plays an important role in the secondary neuronal damage after traumatic brain injury (TBI). Inhibition of histone deacetylases (HDACs) has been shown to reduce reactive oxygen species (ROS) production and NADPH oxidases (Nox) transcription. Vorinostat is an HDAC inhibitor. This study investigated the influence of vorinostat on neurological impairments in a rat model of TBI induced by lateral fluid percussion injury (LFPI). Different concentrations of vorinostat (5, 25, and 50 mg/kg) were administered via intraperitoneal injection. Neurological deficits were evaluated by modified neurological severity scoring (mNSS). Evans blue extravasation was performed to assess blood–brain barrier (BBB) permeability. Morris water maze assay was performed to evaluate cognitive impairments. Protein levels were evaluated through ELISA and Western blot. Vorinostat was found to attenuate TBI induced brain edema and BBB permeability in rats. Vorinostat also alleviated TBI-induced neurological impairments and anxiety-like behavior in rats. Vorinostat attenuated TBI induced apoptosis and oxidative stresses in ipsilateral injury cortical tissue. Vorinostat inhibited HDAC1, HDAC3, and Nox4 while activated AMPK signaling in ipsilateral injury cortical tissue. In conclusion, administration of vorinostat alleviates the secondary damage of TBI in rat model. The oxidative stress in the ipsilateral injury cortical tissues is decreased by the inhibition of Nox4 expression and the activation of AMPK.

1. Introduction

Traumatic brain injury (TBI) has a serious impact on human health and is one of the leading causes of death [1]. In China, the annual TBI mortality rate is 12.99 per 100,000 people [2]. Meanwhile in Europe, the number of hospital admissions due to TBI is 287.2/100,000, with a death rate of 11.7% [3]. Although the mortality rate of TBI has decreased in recent years due to improved therapy, a large number of surviving patients still suffer from neurological impairment due to neuronal damage.

The mechanisms of neuronal damage after TBI are complex, and the relative molecular signaling pathways are not clear. Oxidative stress is crucial in the development of inflammation, secondary neuronal damage, and brain edema after TBI [4]. Under pathological conditions after brain injury, the balance between reactive oxygen species (ROS) and antioxidant rapidly alters and affect the

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sensitivity and development of TBI [5].

Histone deacetylases (HDACs) are key regulators of gene expression. The inhibition of HDACs is demonstrated to reduce ROS production and the transcription of NADPH oxidases (Nox) [6]. Cerebral ischemia results in significant upregulation of HDAC1/2/3 expression, and their inhibitors have been shown to effectively reduce the damage caused by cerebral ischemia [7]. Vorinostat is an HDAC inhibitor. It is reported that the treatment of vorinostat improves the damage caused by TBI [8]. Vorinostat also ameliorates the cognitive impairment in a corticosterone-induced chronic stress model [9]. In this study, we administered vorinostat after TBI to explore the protective effect of vorinostat on neurological damage.

2. Methods

2.1. Traumatic brain injury rat model

A total number of 162 adult male Sprague-Dawley rats (300–350 g, aged 8–10 weeks) were used in this study. The traumatic brain injury model was established by lateral fluid percussion injury (LFPI). Animals were anesthetized with isoflurane inhalation, and the skin and periosteum along the brain midline were sliced to expose the right parietal bone. A small hole was opened 3 mm to the sagittal suture and 3.5 mm behind coronal suture, and a 3 atm pressure was executed by hydraulic equipment. In the sham group, rats underwent the same surgical procedure without pressure destruction. All the animal experiments were approved by the animal use committee in the Shanghai East Hospital (#2021-010).

2.2. Experimental procedure

In this research, animals were randomized into different groups. Different concentrations of vorinostat (5, 25, and 50 mg/kg) were administered through intraperitoneal injection [8]. Administration was carried out at 1, 12, and 24 h after TBI, and then once daily. The vehicle control group was administered with saline solution.

Neurological deficit evaluation was performed before TBI and on days 1, 3, 7, and 14 after TBI. Brain edema and Evans blue extravasation were performed on day 3 after TBI. TUNEL assay and Western blot were performed on day 7 after TBI. Water maze test was initiated on day 15 after TBI. Open field test experiment was initiated on day 20 after TBI. The rats were euthanized with sodium pentobarbital.

2.3. Neurological deficit evaluation

Neurological deficit evaluation was performed by modified neurological severity scoring (mNSS) [10,11]. The higher mNSS score shows more severe brain injury. 13–18 points: severe damage; 7–12 points: moderate damage; 1–6 points: mild damage; 0 point: normal function.

2.4. Brain water content

3 days after TBI, brain tissues were collected and weighted to determine wet weight. Dry weight was weighted after the tissues being dried for 24 h at 100 °C. BWC (%) = (wet weight – dry weight)/wet weight × 100.

2.5. Evans blue extravasation

4 mL/kg of 2% Evans blue (Sigma Aldrich, USA) was intravenously injected 2 h before rats were killed. Brain sample was homogenized in 50% trichloroacetic acid solution and diluted with ethanol. The absorbance at 610 nm was evaluated.

2.6. Morris water maze

The assay was composed of cue, spatial, and probe tests and each test lasted for no more than 60 s. The maze was a round pool, which was 120 cm in diameter and 50 cm in depth. A white platform, 6 cm in diameter and 30 cm in height, lay in the southwest quadrant of the pool. The cue test had a visible platform 2 cm below the water level where the rats could stay for 30 s after finding or being guided to the platform. The space test submerged the platform in water. The rats were released and allowed to swim to find the platform. The latency in finding the platform was measured in spatial test. The probe test was performed without platform. Animals were placed facing the wall in each of the 4 quadrants at the beginning and time in target quadrant was recorded.

2.7. Open field test

Animals were housed in an acrylic box. The inner surface of the test apparatus was painted with black.The floor of the apparatus (100 cm × 100 cm × 40 cm) was divided into 25 identical squares (20 cm × 20 cm) with white stripes. In a dark (visibility was 5 m) and quiet room, a single rat was placed at the center of the arena and allowed to explore for 5 min. The circuit breaks number was counted as locomotion counts and the time in central area were recorded. After each of these tests, the excrements were cleaned and the arena was dealt with 75% alcohol.

2.8. TUNEL

In this research, TUNEL staining was performed by in situ Cell Death Detection Kit (Roche, Germany). Brain sections were dewaxed and incubated with TUNEL reactant at 37 °C for 1 h. Apoptotic cells were observed and recorded under microscope in six randomly selected visual fields from each section. The apoptosis rate was calculated by the formula: apoptotic cells/all cells in a field × 100%.

2.9. Oxidative stress analysis

In this research, the levels of glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) in the lesioned cortices tissues were determined by relative ELISA kits (Nanjing Jiancheng Bioengineering, China).

2.10. Western blot

Western blot was performed by standard protocol. Antibodies used in this research included anti-Nox4 (Abcam, USA), anti-HDAC1 (Cell Signaling Technology, USA), anti-HDAC3 (Cell Signaling Technology), anti-AMP-activated protein kinase (AMPK) (Abcam), anti-p-AMPK (Abcam), and anti-GAPDH (Abcam).

2.11. Statistical analysis

Statistical analysis was performed by SPSS 17.0 software. All data were expressed as mean ± SD. Two-way ANOVA followed Tukey's multiple comparisons test or one-way ANOVA followed Dunn's multiple comparisons test were used for statistical comparisons. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Vorinostat attenuates TBI induced brain edema and the permeability of BBB in rats

Three days after TBI injury, the edema in different sites was examined following administration of different concentrations of vorinostat. Brain water contents in the injury ipsilateral and contralateral cortex were compared between different groups. Brain water contents in the injury ipsilateral cortex of the vehicle group was dramatically higher than that of the VOR groups and the sham group (Fig. 1A; Sham: 78.34 ± 0.74, Vehicle: 85.35 ± 1.36, VOR50: 80.31 ± 1.35, in %). However, there was no significant difference in brain water content in the contralateral cortex among these groups (Fig. 1B). Evans blue extravasation was performed 3 days after TBI.
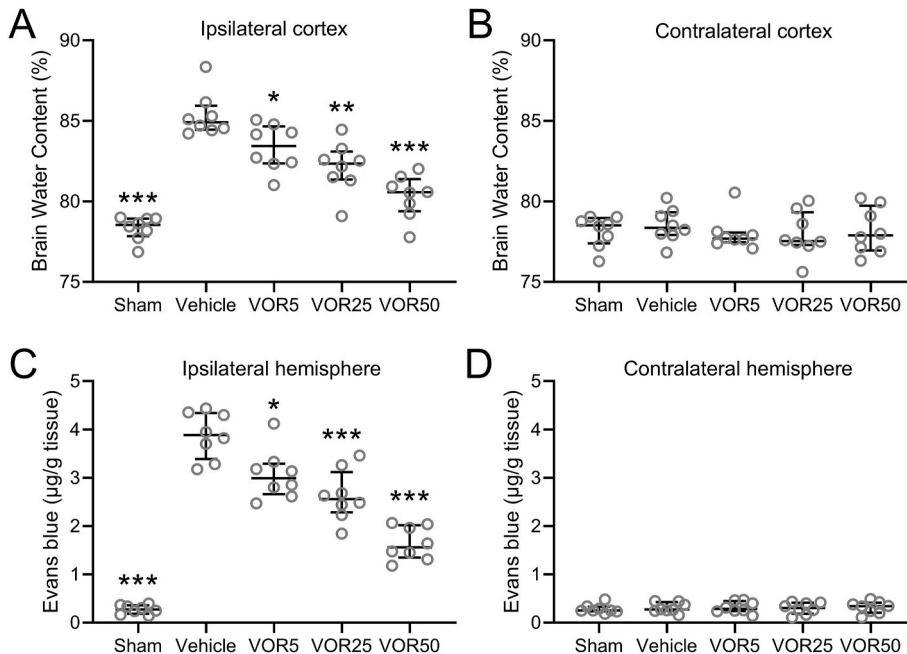

Fig. 1. Vorinostat attenuated TBI induced brain edema and Evans blue extravasation in rats. Brain water contents at injury ipsilateral and contralateral cortex were compared 3 days after TBI (A and B). BBB permeability in ipsilateral injury hemisphere (C) and contralateral un-injury hemisphere (D) was measured 3 days after TBI by Evans blue extravasation. The data were shown as median (interquartile range). N = 8 for each group. One-way ANOVA followed Dunn's multiple comparisons test. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to vehicle group.

In Fig. 1C (Sham: 0.274 ± 0.09, Vehicle: 3.88 ± 0.47, VOR50: 1.64 ± 0.35, in μg/g tissue), in ipsilateral injury hemisphere, the level of Evans blue in the vehicle group was significantly higher than that in the other groups. In contralateral un-injury hemisphere, the level of Evans blue showed no significant difference (Fig. 1D).

3.2. Vorinostat attenuates TBI induced neurological impairments and anxiety-like in rats

Neurological impairment was evaluated by mNSS. mNSS was significantly elevated by TBI in the vehicle group and the VOR groups when compared with the sham group, but mNSS in VOR groups were significantly lower than in the vehicle group at 14 days post-TBI (Fig. 2A). In Fig. 2B, the vehicle group had a significant shorter time spent in the central area than other groups.

3.3. Vorinostat attenuates TBI caused cognitive impairment

After 4 trails of Morris water maze test, the rat's escape latencies were significantly higher in the vehicle group than in the sham group and the VOR groups (Fig. 3A). The vehicle group had lower average swim speed (Sham: 26.72 ± 3.64, Vehicle: 14.38 ± 4.19, VOR50: 23.18 ± 4.15, in cm/s), platform site crossing number (Sham: 6.75 ± 1.04, Vehicle: 2.13 ± 0.99, VOR50: 4.88 ± 1.46, in numbers), and spent less time in the target quadrant in 60 s than the sham group, the VOR25 group, and the VOR50 group (Fig. 3B–D).

3.4. Vorinostat attenuates TBI induced cell apoptosis

Cell apoptosis in lesioned cortices tissue was analyzed through TUNEL staining. In the vehicle group, the ratio of TUNEL positive cell was dramatically higher than in the sham group and the VOR50 group (Fig. 4; Sham: 1.91 ± 0.64, Vehicle: 28.87 ± 5.11, VOR50: 14.23 ± 4.12, in %).

3.5. Vorinostat attenuates TBI induced oxidative stress in the ipsilateral injury cortical tissues of experimental rats

The levels of oxidative stress indicators MDA, SOD, and GSH in the lesioned cortices tissues were examined 7 days after TBI. MDA level in the vehicle group was significantly higher than in the sham group and the VOR50 group (Sham: 3.39 ± 0.95, Vehicle: 24.93 ± 5.41, VOR50: 11.55 ± 3.91, in nmol/mg tissue), while the levels of SOD (Sham: 38.36 ± 6.39, Vehicle: 11.57 ± 5.12, VOR50: 28.96 ± 6.21, in nmol/mg tissue) and GSH (Sham: 123.42 ± 15.12, Vehicle: 56.67 ± 18.48, VOR50: 87.68 ± 16.99, in pmol/mg tissue) were significantly lower (Fig. 5A–C).

3.6. Vorinostat inhibits HDAC1, HDAC3, Nox4 but activates AMPK signaling in the ipsilateral injury cortical tissues of experimental TBI rats

In Fig. 6A–D, HDAC1, HDAC3, and Nox4 levels in the vehicle group were significantly higher than in the other groups. However, the vehicle group had significant a lower phosphorylation level of AMPK than the other groups (Fig. 6A and E).

4. Discussion

TBI survivors suffer from physical, mental, and cognitive disabilities that influence their quality of life and impose a heavy burden and cost on society [12]. Moreover, disabilities are not limited to severe TBI patients [5].
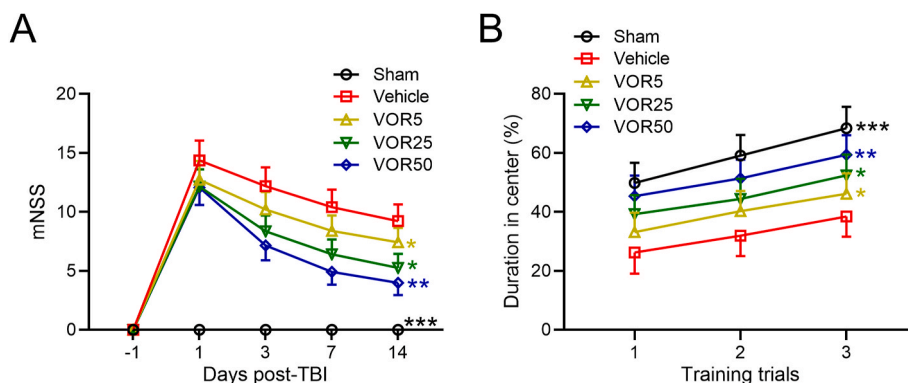

Fig. 2. Vorinostat attenuated TBI induced neurological impairments and anxiety-like in rats. Neurological deficit scores were measured pre, 1, 3, 7 and 14 days post-TBI (A). The time spent in the central area of the open field test was recorded (B). N = 8 for each group. The data were shown as median (interquartile range). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to vehicle group. Two-way ANOVA followed Tukey's multiple comparisons test.
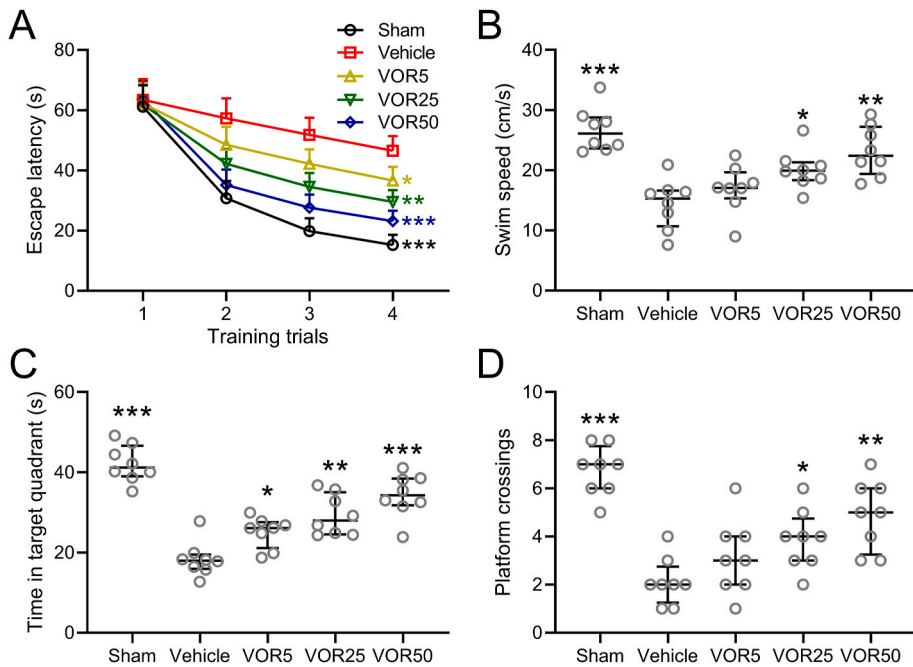
Fig. 3. Vorinostat attenuated TBI induced cognitive impairments in rats. In 4 trails of Morris water maze test, the rat's escape latencies (A) and the average swim speed (B) were measured. In the probe trial, the time in the target quadrant in 60 s (C) and the number of platform site crossings (D) were recorded. N = 8 for each group. The data were shown as median (interquartile range). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to vehicle group. Two-way ANOVA followed Tukey's multiple comparisons test and One-way ANOVA followed Dunn's multiple comparisons test.
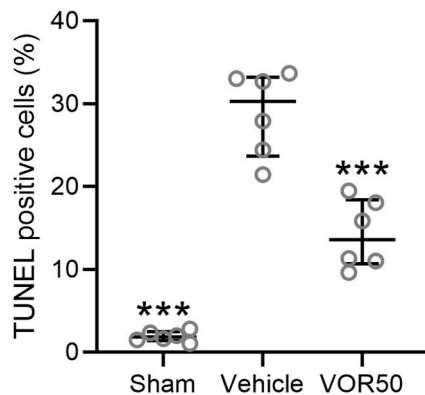

Fig. 4. Vorinostat attenuated TBI induced cell apoptosis in the lesioned cortices tissues of experimental rats. The ratios of TUNEL positive cells in different groups were shown. The data were shown as median (interquartile range). One-way ANOVA followed Dunn's multiple comparisons test. ***$p < 0.001$ compared to vehicle group.

After the primary mechanical injury of TBI, the molecular mechanisms of secondary injury become more complex [13]. This complicates the search for appropriate TBI therapeutic strategies [14]. Secondary pathologic mechanisms include neuroinflammation, ischemia, edema, and hypoxia [15]. These symptoms progress after injury and lead to neuronal death in injury tissue (even extending to initially healthy surrounding tissue) [16].

Oxidative stress plays a significant role in the secondary damage of TBI pathology [17,18]. Pathological conditions after brain injury significantly alter the balance between ROS and antioxidants, which affects the sensitivity and progression of TBI [19,20]. Therefore, inhibiting ROS creation may be an effective way to treat TBI.

Histone acetylation is a crucial mechanism that regulates gene expression. Elevated histone acetylation is usually associated with enhanced gene transcription, while the deacetylation of histone is usually associated with inhibited gene expression [21,22]. HDACs are key regulators of gene expression, removing acetyl groups from histones through their enzymatic activity [23].

HDAC inhibitors have been used in neurology, particularly in psychiatry and neurodegenerative diseases [24]. A study has shown that the inhibition of HDAC can promote functional recovery and neuronal reorganization after brain injury by increasing BDNF
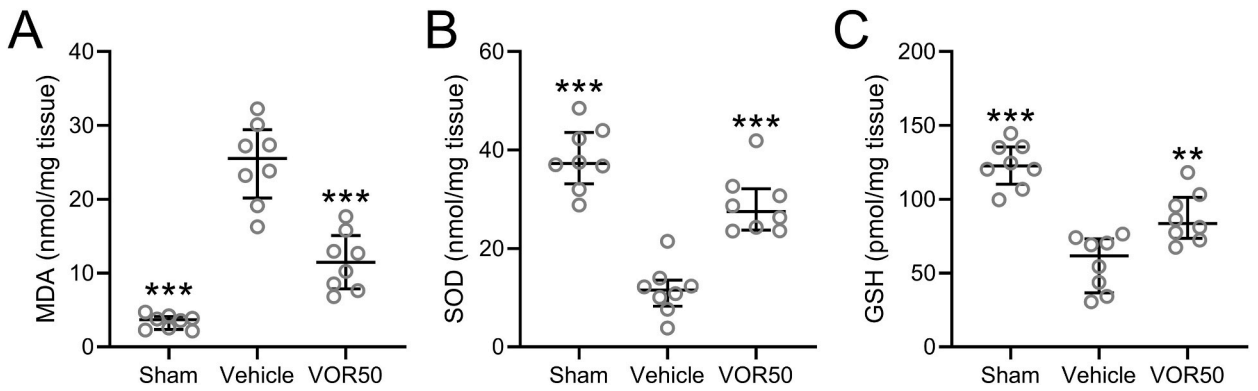
Fig. 5. Vorinostat attenuated TBI induced oxidative stresses in the ipsilateral injury cortical tissues of experimental rats. The levels of oxidative stress indicators MDA (A), SOD (B), and GSH (C) in the lesioned cortices tissues 7 days after TBI were examined. N = 8 for each group. The data were shown as median (interquartile range). One-way ANOVA followed Dunn's multiple comparisons test. **p < 0.01 and ***p < 0.001 compared to vehicle group.
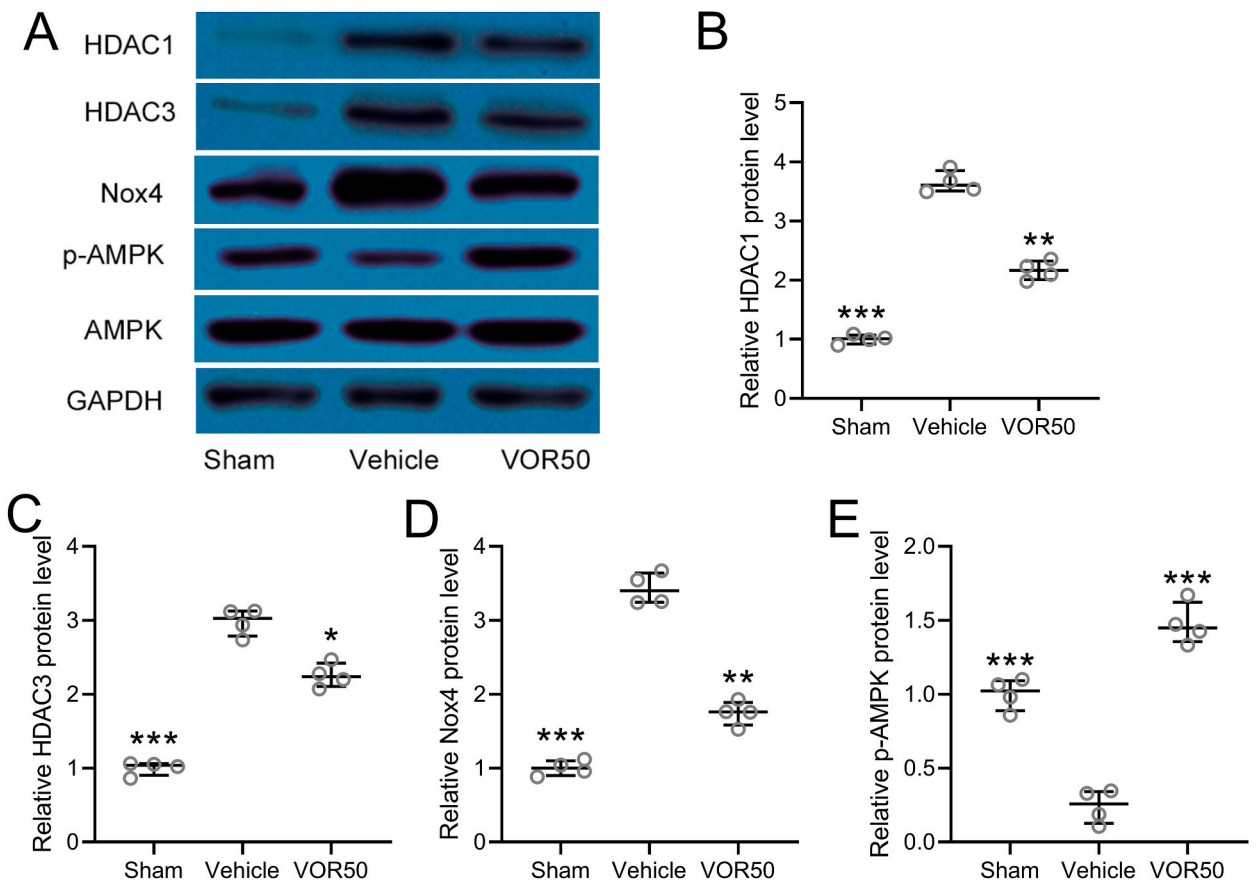

Fig. 6. Vorinostat inhibited HDAC1, HDAC3, Nox4 but activated AMPK signaling in the ipsilateral injury cortical tissues of experimental TBI rats. Western blotting was used to measure the protein expressions of HDAC1, HDAC3, Nox4, *p*-AMPK, and AMPK in the lesioned cortices tissues 7 days after TBI (A). GAPDH was used as a loading control. The original uncropped blot images are shown in Figure S1. The expressions were normalized to sham (B–E). N = 4 from 8 rats in each group. The data were shown as median (interquartile range). One-way ANOVA followed Dunn's multiple comparisons test. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to vehicle group.

expression [25]. HDAC inhibitors increased synaptic bouton number in rewiring corticospinal fibers and enhanced motor function after TBI [25]. There is growing evidence that HDAC inhibitors have neuroprotective effects in different lesions of the central nervous system [26,27]. Furthermore, HDAC inhibitors have been shown to significantly reduce the production of ROS [6].

HDAC inhibitors have been used as targeted drugs in oncology therapy, and their safety has been clearly demonstrated [28]. Although HDAC inhibitors have been shown to promote neuronal growth in various insults to the CNS, their application to TBI has not been reported. This research explored the effects of HDAC inhibitor vorinostat on the secondary damage caused by TBI and its molecular mechanism.

The degree of edema in different brain sites was compared three days after TBI injury following administration of different concentrations of vorinostat. Vorinostat was found to be effective in reducing brain edema in a dose-dependent manner. The results of Evans blue extravasation assays proved that vorinostat could improve the integrity of BBB in rat TBI model. Neurological dysfunction was detected at different time points after TBI injury following administration of different concentrations of vorinostat. The open field test was performed 20 days after TBI, and vorinostat was found to improve neurological damage, as well as improve anxiety symptoms in rats. The water maze test after 15 days revealed that vorinostat was able to improve cognitive impairment in rats. TUNEL staining identified apoptosis in cortical tissue at the TBI injury and found that vorinostat was able to significantly inhibit apoptosis. Thus, the administration of vorinostat alleviated the symptoms caused by TBI in rat model in a dose-dependent manner.

Nox family is enzymes whose main function is to produce ROS [29]. Many researchers have begun to focus on the pathological function of Nox enzymes in brain injury. Evidence suggests that chronic Nox activation exacerbates primary injury, and Nox enzymes are potential targets for TBI therapy [30,31]. Among the subtypes of Nox enzymes, Nox2 is the most studied in the pathogenesis of TBI, but recent studies support a new role for Nox4 in this process [32]. In immune cells, multiple HDAC inhibitors significantly reduced Nox2 expression in a dose-dependent manner, while decreasing superoxide production [33]. HDAC inhibition also reduced the expression of Nox 1, Nox4, and Nox 5 [34].

In this research, we proved that vorinostat was able to reduce the oxidative stress that damages the ipsilateral cerebral cortex tissue. In TBI rat model, the protein levels of HDAC1 and HDAC3 were both significantly elevated. Increased HDAC1 and HDAC3 levels in TBI rat model could be decreased by the administration of vorinostat. Meanwhile, increased protein level of Nox4 in TBI rat model was also decreased by vorinostat.

The AMPK pathway is an important pathway that regulates intracellular redox and ROS homeostasis [35]. HDAC inhibitors can effectively activate AMPK and inhibit Nox enzyme transcription to limit the production of ROS [36]. In this research, the administration of vorinostat also enhanced the phosphorylation of AMPK in TBI rat model. Therefore, we proposed that the use of HDAC inhibitor vorinostat after TBI could delay the secondary damage by activating AMPK.

There are several potential limitations deserving attention in our experiments. First, we investigated the effects of vorinostat against brain damage after TBI by the inhibition of Nox4, but more details in this molecular mechanism remain unclear. Second, we did not measure the compound in the brain. Thus, it is not possible to verify whether vorinostat was acting or at an effective concentration at the proposed site of action. Third, only male rats were used in this study, as female rats might be affected by hormone levels, which will then affect the behavior of animals accordingly, resulting in unreliable experimental data. However, future studies may include female animals to see if similar findings can be obtained. Last, the sample size of this study was small. We will increase the sample size in our future studies.

5. Conclusion

In conclusion, the administration of vorinostat alleviates the secondary damage of TBI in rat model. The oxidative stress in the ipsilateral injury cortical tissues is decreased by the inhibition of Nox4 expression and the activation of AMPK.

Ethical statement

All the animal experiments were approved by the animal use committee in the Shanghai East Hospital (#2021-010).

Author contribution statement

Yiming Lu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yiming Chen; Siyi Xu; Yanfei Zhang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Liang Wei; Wei Chen; Min Liu; Chunlong Zhong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e18485.

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