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Valproic acid increases white matter repair and neurogenesis after stroke

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Abstract

Acute treatment of stroke with HDAC inhibitors has been shown to reduce ischemic cell damage; however, it is unclear whether delayed treatment with HDAC inhibitors will contribute to the brain repair and plasticity. In the present study, we investigated the effects of delayed treatment of stroke with a pan HDAC inhibitor, valproic acid (VPA), on white matter injury and neurogenesis during stroke recovery. Administration of VPA at a dose of 100 mg/kg for 7 days starting 24 hours after middle cerebral artery occlusion (MCAo) in rats significantly improved neurological outcome measured 7 to 28 days post-MCAo. In addition, the VPA treatment significantly increased oligodendrocyte survival and newly generated oligodendrocytes, which was associated with elevation of myelinated axonal density in the ischemic boundary 28 days after MCAo. VPA treatment also increased the expression of glutamate transporter 1 (GLT1) in the ischemic boundary after stroke, and increased acetylated histone H4 expression in neuroblasts and the number of new neurons in striatal ischemic boundary region. This study provides new evidence that the delayed VPA treatment enhances white matter repair and neurogenesis in ischemic brain, which may contribute to improved functional outcome.

Keywords

Valproic acid; oligodendrocyte; axon; neural progenitor cells; subventricular zone (SVZ); stroke

INTRODUCTION

Histone deacetylases (HDACs) are enzymes involved in epigenetic modifications that shift the balance toward chromatin condensation and silencing of gene expression. Treatment of acute stroke with HDAC inhibitors, such as VPA and suberoylanilide hydroxamic acid (SAHA), reduces ischemic neuronal damage and improves neurological outcome (Ren et al., 2004, Faraco et al., 2006, Kim et al., 2007, Sinn et al., 2007, Lv et al., 2011, Wang et al., 2011). In contrast to their roles in neuroprotection, the effect of HDAC inhibitors on brain repair has not been extensively studied during stroke recovery.

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Oligodendrocytes are myelin-forming cells which support axon integrity in the central nervous system (Baumann and Pham-Dinh, 2001, Emery, 2010). Mature oligodendrocytes are derived from non-myelinating oligodendrocyte progenitor cells (OPCs). HDAC activity is essential for oligodendrocyte differentiation in the developing rodent brain (Marin-Husstege et al., 2002, Shen et al., 2005, Lyssiotis et al., 2007, Ye et al., 2009, Chen et al., 2011, Jacob et al., 2011). Inhibition of HDACs with pharmacological inhibitors or ablation of HDAC1 and HDAC2 genes suppresses oligodendrocyte differentiation (Marin-Husstege et al., 2002, Shen et al., 2005, Lyssiotis et al., 2007, Ye et al., 2009, Swiss et al., 2011). However, a recent *in vitro* study shows that HDAC inhibition by SAHA preserves mature oligodendrocytes and promotes functional recovery of axons in mouse optic nerve after oxygen and glucose deprivation (OGD) (Baltan et al., 2011). Oligodendrocytes are highly vulnerable to ischemic insult (Pantoni et al., 1996, Petito et al., 1998, Dewar et al., 2003). Regeneration of mature oligodendrocytes has been observed in the peri-infarct areas after stroke (Gregersen et al., 2001, Tanaka et al., 2003). Stroke induces oligodendrogenesis (Mandai et al., 1997, Mabuchi et al., 2000, Gregersen et al., 2001, Tanaka et al., 2001, Dewar et al., 2003) and neurogenesis (Parent et al., 2002, Jin et al., 2004, Zhang et al., 2004a, Zhang et al., 2004b) that are involved in brain repair. The effect of HDAC inhibitors on oligodendrocytes in ischemic brain is not known. In the present study, using a rat model of focal cerebral ischemia, we investigated the effect of a pan-HDAC inhibitor, VPA, on white matter injury and neurogenesis, when VPA was administered one day after ischemic stroke.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Henry Ford Health Systems.

Model of middle cerebral artery occlusion (MCAo)

Adult male rats (3-4 months) were employed in this study. Permanent right MCAo was induced by advancing a 4-0 surgical nylon suture with an expanded tip from the external carotid artery into the lumen of the internal carotid artery to block the origin of the MCA (Mao et al., 1999).

Experimental protocol

To examine the effect of VPA on neurological outcome, VPA powder was dissolved in saline and VPA at a dose of 100 mg/kg was administered (i.p) daily for 7 consecutive days starting 1 day after MCAo (n=5). The dose of VPA was selected based on a published study showing that this dose inhibits HDAC activity *in vivo* (Loscher et al., 1993, Bredy and Barad, 2008). Ischemic rats treated with saline were used as a control group (n=10). Rats subjected to MCAo were randomly assigned to the VPA group or saline group. Randomisation was performed by using computer software-generated random allocation (GraphPad Software). All rats were killed 28 days after MCAo.

Behavioral tests

An array of behavioral tests to detect sensorimotor deficits were performed 1, 7, 14, 21, 28 days after onset of MCAo by observers blinded to the treatments.

Modified neurological severity score (mNSS) (Zhang et al., 2002) : Rats were tested for motor, sensory, reflex, and balance dysfunction. Neurological function was graded on a scale of 0 to 18 (normal score, 0; maximal deficit score, 18).

Foot-fault test: A modified foot-fault test was employed to measure forelimb placement dysfunction (Zhang et al., 2002). The total number of steps (movement of each forelimb) that the rat used to cross the grid and the total number of foot faults for the left forelimb were recorded.

Adhesive removal test: The adhesive removal test measures somatosensory deficits in rats after stroke (Schallert and Whishaw, 1984). The mean time (seconds) required to remove the stimulus from the left limb was recorded.

Measurements of infarct volume

Rat brains were fixed by transcardial perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde, and the brains were cut into seven equally spaced (2 mm) coronal blocks and embedded in paraffin. Brain coronal sections (6 μ m thickness) was cut from each block and stained with Hematoxylin and Eosin (H&E). Infarction volume was measured on seven coronal sections using a microcomputer imaging device (MCID) image analysis system (Imaging Research, St. Catharines, Canada). The indirect infarct area, in which the intact area of the ipsilateral hemisphere was subtracted from the area of the contralateral hemisphere, was calculated. The ischemic volume is presented as the percentage of infarct volume of the contralateral hemisphere (Zhang et al., 1997a, Zhang et al., 1997b).

BrdU labeling *in vivo* and TUNEL

To label proliferating cells, all rats were treated with a daily injection of BrdU (100 mg/kg, i.p) for 6 consecutive days starting 1 day after MCAo. For immunolabeling with anti-BrdU antibodies, after the completion of the first staining, the cells were then treated with 2N HCl for 10 min at 37° in order to denature DNA, followed by equilibration in 0.1 M sodium borate, pH 8.6, for 10 min. The primary antibody for anti-BrdU (Dako, Carpinteria, CA, USA) was used at 1:100 dilution in PBS containing 1% BSA and 0.5% Triton X-100 overnight at 4°, followed by Cy3-conjugated whole-Ig secondary antiserum. Cells were then counterstained with DAPI for nuclei visualization. The identification of apoptotic cells was performed using the ApopTag plus kit (Chemicon, CA, USA) following the manufacturer's manual.

Histopathology and immunohistochemistry

A series of coronal sections (6 μ m thick) were obtained at the centre of the lesion, corresponding to bregma -1~1mm. Five sections (~100 μ m apart) were used for immunohistochemistry. The following primary antibodies were used: mouse anti-BrdU (1:1,00; Boehringer Mannheim, Indianapolis, IN, USA), a marker of proliferating cells, mouse anti- β -III tubulin1 (TuJ-1, 1:500; Covance, MI, USA), a marker of neuroblasts, rabbit anti-gial fibrillary acidic protein (GFAP, 1:500; Dako), a marker of astrocytes, goat anti-doublecortin (DCX, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA), a marker of neuroblasts, mouse anti-2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase, 1:200, Chemicon) and rat anti-myelin basic protein (MBP, 1:50, Abcam, Cambridge, MA, USA), markers for oligodendrocytes (OLGs), rabbit anti-chondroitin sulfate proteoglycan (NG2, 1:800, Chemicon), a marker of oligodendrocyte progenitor cells (OPCs), and chicken anti-neurofilament-H (NF-H, 1:10,000, ABR Affinity Bioreagents, Golden, CO, USA), a marker of axons, mouse anti-histone H3 (1:100, Pharmingen, San Jose, CA, USA), mouse anti-histone H4 (1:100, Pharmingen), guinea pig anti-glutamate transporter 1 (GLT1, 1:500, Chemicon), mouse anti-synaptophysin (1:500, Abcam), goat anti-sox2 (1:100, Santa Cruz Biotechnology). Nonspecific binding sites were blocked with PBS with 1% bovine serum albumin goat serum for 1 h at room temperature. The tissues were then incubated with the primary antibodies listed above and with CY3 or FITC -conjugated secondary antibodies.

Nuclei were counterstained with 4', 6'-diamidino-2-phenylindole (DAPI) (1:10000, Vector Laboratories, Burlingame, CA, USA). Bielschowsky and Luxol (B/L) Fast Blue staining were used to identify myelinated axons (Zhang et al., 2010). Control experiments consisted of staining brain coronal tissue sections as outlined above, but omitting the primary antibodies.

Image acquisition and Quantification

Coronal sections were digitized using a 40× objective using a three-CCD color video camera (Sony DXC-970MD) interfaced with an MCID system. For quantification of axon and myelinating OLs, the density of NF-H, B/L Blue staining, MBP immunoreactive area throughout the SVZ, peri-infarct corpus callosum and striatum, and the contralateral homologous area were digitized. Two coronal sections (100 μm interval) from the center of the ischemic core (bregma 0.2 mm) were used per staining from each animal. For quantification of axon and myelinating OLs, NF-H, B/L Blue staining and CNPase immunoreactive areas were measured in five fields of view within peri-infarct corpus callosum and striatum. In preliminary data analysis, we quantified MBP and NF-H immunoreactive areas in the contralateral hemisphere of sham-operated animals and of ischemic animals treated with saline or VPA. We did not find a significant difference of the immunoreactive areas in the contralateral hemisphere among these three groups (Fig. 1A and B). Thus, the contralateral hemisphere was used as the internal control.

Statistical Analysis

Data including behavioral tests and cell countings were evaluated by an investigator blinded to the treatment status of each animal. Data were evaluated for normality and the data transformation was performed if data were not normal. As a result, the behavior tests were not normal, and ranked data were used for the analysis. The repeated measure of ANOVA was used to test the group difference on functional recovery measured from multiple behavior tests. Two-sample t-test was used to test the effect of VPA on cell measurements, compared to controls. Chi-Square test was done to compare the mortality rate. Results are presented as mean ± SE for data illustration. A value of $p < 0.05$ was taken as significant.

RESULTS

VPA improves neurological outcome

To examine the effect of VPA on neurological outcome, we performed an array of behavioral tests. All rats exhibited neurological deficits 24 h after MCAo (Fig. 2A-C). The mortality rates in the control and VPA-treated groups were 23% and 28.5%, respectively, which is not statistically significant ($p > 0.05$). These rats were excluded from the study. Treatment of ischemic rats with VPA initiated 24 h after MCAo significantly improved neurological outcome starting 7 days after MCAo compared to rats treated with saline (Fig. 2). The improved neurological outcome persisted for at least 28 days, the end time point of the present study (Fig. 2). In contrast, VPA treatment did not significantly ($p > 0.05$) reduce infarct volume ($32.1\% \pm 7.5\%$, $n=5$) compared to the volume in the saline group ($30.9\% \pm 5.6\%$, $n=5$). These data suggest that the effect of VPA on neurological outcome is independent of the infarct volume.

VPA increases OLG survival and promotes OPC differentiation into OLGs

Immunostaining revealed that ischemic rats treated with VPA (Fig. 3A and B) exhibited a significant ($p < 0.01$) increase in MBP immunoreactivity along the striatal peri-infarct region compared to the ischemic rats treated with saline (Fig. 3A and B). No significant difference of MBP immunoreactivity was observed in the contralateral hemisphere between VPA

($10.8 \pm 1.2\%$, $n=5$) and saline ($10.3 \pm 1.6\%$, $n=5$, $p > 0.05$) treated groups. In addition, VPA treatment significantly increased the number of CNPase positive cells in the striatal peri-infarct region (Fig. 3C and D). Both MBP and CNPase are markers of oligodendrocytes (Baumann and Pham-Dinh, 2001). Thus, these data suggest that VPA increases the number of OLGs after stroke.

To examine whether VPA reduces apoptotic cell death, leading to increases in OLGs, we measured the number of TUNEL positive cells. Our data demonstrated that the administration of VPA significantly reduced the number of CNPase and TUNEL positive cells in the ischemic striatum compared with the saline-treated control group (Fig. 3C, E and F), suggesting that VPA reduces apoptotic OLG death.

Mature OLGs are derived from non-myelinating OPCs because OLGs do not proliferate (Baumann and Pham-Dinh, 2001). OPCs in adult brain contribute to replenishment of OLGs after ischemic insults (Zhang et al., 2009, Iwai et al., 2010, Zhang et al., 2010). Thus, the increase in OLGs by VPA could result from generation of new OLGs. Accordingly, we measured the number of NG2 positive cells, a marker of OPCs, and found that VPA treatment did not significantly affect the number of NG2 positive cells in the striatal peri-infarct region ($1.1 \pm 0.1\%$ vs $1.2 \pm 0.1\%$ in saline group, $n=5$, $p=0.34$), the corpus collosum ($1.1 \pm 0.1\%$ vs $1.2 \pm 0.1\%$ in saline group, $p=0.35$) and the SVZ ($1.1 \pm 0.1\%$ vs $1.4 \pm 0.3\%$ in saline group, $p=0.4$). We then examined the number of BrdU and CNPase positive cells that represent newly generated OLGs (Zhang et al., 2010). Double immunofluorescent staining revealed that VPA treatment significantly increased the number of BrdU and CNPase positive cells in the striatal peri-infarct zone compared with the cells in the saline group (Fig. 4A, B and F). Also, VPA injection significantly increased the number of CNPase positive cells in SVZ after stroke, compared with the saline treated group (Fig. 4C, D and F). These data suggest that VPA promotes OPCs to differentiate into mature OLGs.

GLT-1 by clearance of glutamate reduces ischemic grey and white matter damage (Harvey et al., 2011) and HDAC inhibition upregulates GLT-1 expression (Baltan et al., 2011). We therefore examined whether VPA affects GLT1 expression. Immunohistochemistry data showed a substantial elevation of GLT-1 immunoreactivity in peri-infarct region of rats treated with VPA compared to rats treated with saline control group (Fig. 4G, H and J). Double immunostaining revealed that GLT1 immunoreactivity was synaptophysin positive (Fig. 4I) and that some of GLT1 positive cells were GFAP positive (Fig. 4H). These data suggest that inhibition of HDAC by VPA increases GLT-1 levels.

VPA attenuates axonal loss

Ischemia directly damages axons (Carmichael, 2003, Lo et al., 2003). OLGs myelinate axons and support axonal integrity (Baumann and Pham-Dinh, 2001, Emery, 2010). To examine whether increased OLGs by VPA are related to axonal changes, we performed immunostaining to identify NF-H and Bielschowsky and Luxol fast blue staining to detect myelinated axons. Ischemic rats treated with VPA exhibited significant increases in NF-H immunoreactivity in the white matter compared to rats in the control group 28 days after MCAo (Fig. 5A and B). In addition, treatment with VPA also increased Bielschowsky/Luxol fast blue staining in ischemic boundary zone (Fig. 5C and D). These data indicate that VPA increases axonal density in the ischemic boundary zone.

VPA injection promotes neurogenesis

HDAC inhibition induces neurogenesis after stroke (Kim et al., 2009). Consistently, we found that VPA treatment substantially increased the number of DCX, a marker of neuroblasts, and BrdU positive cells in the SVZ and ischemic boundary zone (Fig. 6A-F).

DCX positive cells in rats treated with VPA had significantly longer neurite processes than DCX cells in rats treated with saline 28 days after stroke (Fig. 6G), suggesting that these neuroblasts are becoming mature neurons (Yamashita et al., 2006). VPA is a pan-HDAC inhibitor. HDAC inhibition affects the acetylation status of histone proteins within chromatin. To examine whether VPA affects levels of histone H4 acetylation, we performed double immunostaining to detect acetylated-Histone H4 in neuroblasts and neural progenitor cells. We found that VPA treatment increased the number of acetylated-Histone H4 positive and DCX positive neuroblasts in ipsilateral SVZ (Fig. 7A and D) and ischemic boundary zone (Fig. 7B and D) as well, compared with saline control group. In addition, many of the acetylated-Histone H4 positive cells were SOX2 positive, a marker of neural progenitor cells (Fig. 7C and E). VPA treatment did not significantly affect acetylated-Histone H4 levels in neuroblasts and neural progenitor cells in the contralateral SVZ (data not shown).

DISCUSSION

The present study demonstrates that administration of VPA starting 24h after stroke improved neurological outcome, which was associated with increases in oligodendrocytes, myelinated axons, and neurogenesis in the peri-infarct region 28 days after stroke. These data suggest that in addition to neurogenesis, VPA has beneficial effects on white matter repair in ischemic brain. Mature oligodendrocytes in adult rodent brain are derived from non-myelinating OPCs that are present in the corpus callosum, the striatum, and the SVZ (Marshall et al., 2005, Polito and Reynolds, 2005, Menn et al., 2006). HDAC activity regulates oligodendrocyte differentiation. During the early postnatal stage when myelination forms, blockade of HDAC activity by pan HDAC inhibitors, VPA or TSA, prevents oligodendrocyte differentiation, leading to hypomyelination (Romm et al., 2005, Shen et al., 2005, Ye et al., 2009). However, the effect of the HDAC inhibitors on suppression of oligodendrocyte differentiation is transient and only takes place during the first two postnatal weeks (Shen et al., 2005). Genetic ablation of HDAC1 and HDAC2 in the mouse blocks oligodendrocyte differentiation by activation of the Wnt signaling pathway (Ye et al., 2009). Stroke induces regeneration of mature oligodendrocytes in the peri-infarct areas after stroke (Gregersen et al., 2001, Tanaka et al., 2003, Zhang et al., 2010). MBP myelinates axons (Baumann and Pham-Dinh, 2001). We now present data showing that VPA substantially increased mature oligodendrocytes identified by MBP and CNPase immunoreactivity. Moreover, VPA treatment robustly augmented myelinated axons. Together, our data suggest that under ischemic conditions, VPA enhances white matter repair.

Our data further suggest that reduction of apoptotic oligodendrocyte death measured by TUNEL positive cells and augmentation of newly generated oligodendrocytes assayed by BrdU/CNPase positive cells likely contribute to increased oligodendrocytes after VPA treatment. The present data are consistent with findings from in vitro ischemia that HDAC inhibition by SAHA preserves mature oligodendrocytes and promotes functional recovery of axons in mouse optic nerve after OGD (Baltan et al., 2011). Although the present study did not detect a significant increase in OPCs, our data suggest that VPA amplifies OPCs in the ischemic brain. Based on the fact that mature oligodendrocytes are derived from OPCs, an increase in the number of new born oligodendrocytes (BrdU/CNPase positive cells) observed in the present study may result from increases of proliferating OPCs. Since BrdU labeling was terminated 8 days after stroke and immunostaining was performed at 28 days after stroke, we may have missed the transient amplification effect of VPA on OPCs. However, we can not rule out the possibility that VPA promotes OPC differentiation into mature oligodendrocytes without increases in OPC. Further studies are warranted.

Oligodendrocytes express AMPA/kainate receptor subunits that can be activated by extracellular glutamate leading to oligodendrocyte death (McCarran and Goldberg, 2007, Tekkok et al., 2007). GLT-1 is a glutamate transporter and reduces extracellular glutamate overflow. Overexpression of GLT-1 reduces ischemic grey and white matter damage (Harvey et al., 2011). HDAC inhibition upregulates GLT-1 expression *in vitro* (Baltan et al., 2011). Consistently, our data show that VPA treatment increased the expression of GLT-1 in the peri-infarct region, suggesting that upregulated GLT-1 could contribute to VPA-reduced oligodendrocyte death by clearance of elevated extracellular glutamate after stroke. In addition, GLT-1 forms macromolecular complexes with multiple proteins, including two oligodendrocyte proteins, CNPase and myelin proteolipid protein (Genda et al., 2011). Thus, in addition to its function to eliminate extracellular glutamate, GLT-1 may affect oligodendrocyte differentiation by directly interacting with these myelin genes. However, further studies are warranted to investigate the role of GLT-1 in mediating HDAC inhibitor-induced oligodendrocyte survival and differentiation after stroke.

Our data also show that the delayed VPA treatment substantially increased neuroblasts in the peri-infarct region, indicating that the VPA enhances neurogenesis, consistent with published studies (Kim et al., 2009). BDNF mediates HDAC inhibitor-induced neurogenesis in ischemic brain (Kim et al., 2009). The present study indicates that VPA treatment elevated acetylated histone H4 in neuroblasts, suggesting that modulation of histone acetylation in neuroblasts could be involved in HDAC inhibitor-induced neurogenesis.

In addition to HDAC inhibition, VPA regulates GABA gene expression, sodium channels and NMDA signaling (Yamamoto et al., 1997, Vreugdenhil et al., 1998, Reynolds et al., 2007, Guidotti et al., 2011), all of which could affect white matter repair and neurogenesis (Tsai et al., 2001, Vicini, 2008). Additional *in vivo* studies are needed to investigate the specific effects of VPA on HDAC inhibition and other molecules.

Together, the present study suggests that the delayed VPA treatment enhances white matter repair and neurogenesis, which could contribute to improved neurological outcome in a rat model of stroke.

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LIST OF ABBREVIATIONS

BrdU	bromodeoxyuridine
CNPase	2'',3''-cyclic nucleotide 3'-phosphodiesterase
DAPI	4',6'-diamidino-2-phenylindole
DCX	doublecortin
GFAP	glial fibrillary acidic protein
GLT1	glutamate transporter 1
HDAC	histone deacetylase
MBP	myelin basic protein
MCAo	middle cerebral artery occlusion

mNSS	modified neurological severity score
NF-H	neurofilament-H
NG2	chondroitin sulfate proteoglycan
OGD	oxygen and glucose deprivation
OLGs	oligodendrocytes
OPCs	oligodendrocyte progenitor cells
SVZ	subventricular zone
Tuj1	β -III tubulin1
VPA	valproic acid

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Highlights

- We assessed the effect of HDAC inhibitor-VPA on post-stroke recovery.
- The administration of VPA starting 24h after stroke improved neurological outcome.
- The VPA treatment enhances white matter repair and neurogenesis after stroke.
- The upregulation of GLT-1 by VPA may lead to increased oligodendrocyte survival.

Figure 1.

Panels A and B showed that there is no significant difference of MBP ($p>0.05$) and NFH ($p>0.05$) immunoreactive cells in contralateral hemisphere of sham-operated animals (sham control, $n=4$) and of ischemic animals treated with saline (control, $n=5$) or VPA (VPA, $n=5$).

Figure 2.

The effect of VPA on neurological outcome. Panels A to C show temporal profiles of foot-fault test (A), adhesive-removal test (B), and mNSS (C) during 28 days after MCAO. Values are mean $\pm$ SE. * $p < 0.05$, # $p < 0.01$ as compared with the saline control group. $n = 10$ / saline control group, $n = 5$ / VPA group.

Figure 3.

The effect of VPA on oligodendrocytes. Panel A shows MBP immunoreactivity in striatal peri-infarct region of representative rat treated with saline (control) or VPA (VPA). Panel B shows quantitative data of MBP density in peri-infarct region 28 days after MC Ao. n=5) exhibited a significant ($p < 0.01$) increase in MBP immunoreactivity along the peri-infarct striatum compared to the ischemic rats treated with saline (A and B, n=5). Panel C shows CNPase (red) and TUNEL (green) positive cells in (green) in striatal peri-infarct region of representative rat treated with saline (control) or VPA (VPA). Panels D to F show quantitative data of CNPase (D), TUNEL (E), and CNPase/TUNEL (F) positive cells in peri-infarct region 28 days after MCAo. n=5 in 100mg/kg VPA-treated group, n=5 in saline control group. * $p < 0.05$, # $p < 0.01$. Scale bar: 20um.

Figure 4.

The effect of VPA on newly generated oligodendrocytes. Panels A-E demonstrates VPA treatment increased the number of CNPase+/BrdU+ in the ischemic striatum (A, B and E), but decreased it in the SVZ (C, D and E) after stroke. Panels F demonstrates that VPA injection significantly increased the number of CNPase positive cells in SVZ after stroke, compared with saline treated cells. Panels G-J show VPA treatment upregulated the expression of GLT1 in the ischemic boundary zone after stroke, which coexpressed with synaptophysin (I) and GFAP (H) positive cells. n=5 in 100mg/kg VPA-treated group, n=5 in saline control group. *p<0.05. Scale bar: 20um.

Figure 5.

VPA attenuates myelinating axonal loss after MCAo. Panels A and B showed a significant higher immunoreactivity to NF-H in the white matter brain of VPA treated animals compared to sham treated animals 28 days after MCAo. Panels C and D show treatment with VPA also increased Bielschowsky/Luxol Fast Blue staining in ischemic boundary zone. * $p < 0.05$. Scale bar: 20 μ m.

Figure 6.

VPA promotes neurogenesis after MCAO. Panels A-E show VPA treatment significantly increased the number of migratory DCX positive cells in both ischemic SVZ (A, B and E) and ischemic boundary zone (C and D) compared with saline-treated control group. Panels A, B and F also show VPA treatment significantly increased the colocalization of DCX and BrdU in ischemic SVZ (A, B and F). The length of dendrite of DCX positive cells were significantly increased after VPA treatment, compared with saline-treated control (G). * $p < 0.05$, LV: lateral ventricle. Scale bar: 20μm.

Figure 7.

VPA increases histone acetylation in neural progenitor cells after MCAO. Panels A, B and D show the accumulation of acetylated-Histone H4 was increased in DCX positive neuroblasts in ipsilateral SVZ (A and D) and IBZ (B and D) treated with VPA, compared with saline control group. Panels C and E show a significant difference of acetylated-Histone H4 in SOX2 positive neural progenitor cells in ipsilateral SVZ compared with that in saline control group.). * $p < 0.05$, $\#p < 0.01$. LV: lateral ventricle. Scale bar: 20um.