Valproic Acid Attenuates Acute Lung Injury Induced by Ischemia–Reperfusion in Rats

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ABSTRACT

Background: Evidence reveals that histone deacetylase (HDAC) inhibition has potential for the treatment of inflammatory diseases. The protective effect of HDAC inhibition involves multiple mechanisms. Heme oxygenase-1 (HO-1) is protective in lung injury as a key regulator of antioxidant response. The authors examined whether HDAC inhibition provided protection against ischemia–reperfusion (I/R) lung injury in rats by up-regulating HO-1 activity.

Methods: Acute lung injury was induced by producing 40 min of ischemia followed by 60 min of reperfusion in isolated perfused rat lungs. The rats were randomly allotted to control group, I/R group, or I/R + valproic acid (VPA) group with or without an HO-1 activity inhibitor (zinc protoporphyrin IX) (n = 6 per group).

Results: I/R caused significant increases in the lung edema, pulmonary arterial pressure, lung injury scores, tumor necrosis factor-α, and cytokine-induced neutrophil chemoattractant-1 concentrations in bronchoalveolar lavage fluid. Malondialdehyde levels, carbonyl contents, and myeloperoxidase-positive cells in lung tissue were also significantly increased. I/R stimulated the degradation of inhibitor of nuclear factor-κB-α, nuclear translocation of nuclear factor-κB, and up-regulation of HO-1 activity. Furthermore, I/R decreased B-cell lymphoma-2, heat shock protein 70, acetylated histone H3 protein expression, and increased the caspase-3 activity in the rat lungs. In contrast, VPA treatment significantly attenuated all the parameters of lung injury, oxidative stress, apoptosis, and inflammation. In addition, VPA treatment also enhanced HO-1 activity. Treatment with zinc protoporphyrin IX blocked the protective effect of VPA.

Conclusions: VPA protected against I/R-induced lung injury. The protective mechanism may be partly due to enhanced HO-1 activity following HDAC inhibition. (Anesthesiology 2015; 122:1327–37)

What We Already Know about This Topic

- Lung injury occurs when perfusion is stopped (40 min) to isolated lungs and then restarted (60 min). Some of these manifestations of lung injury include edema, increased inflammatory cytokines in the airspaces, and up-regulation of heme oxygenase-1 activity.

What This Article Tells Us That Is New

- The administration of valproic acid decreased all the parameters of lung injury, oxidative stress, apoptosis, and inflammation and some of its protection appeared to occur by increasing heme oxygenase-1 activity.
VPA Protects against I/R Lung Injury

Materials and Methods

Preparation of Isolated Perfused Lung

Care of the rats used in this study met the guidelines set forth by the National Institutes of Health (National Academy Press 1996), The National Science Council (Taiwan, Republic of China), and Animal Review Committee of the National Defense Medical Center approved the study protocol. The isolated rat lungs were prepared using previously described methods.8,9 In brief, Sprague–Dawley male rats weighing 350 ± 20 g were anesthetized using intraperitoneal sodium pentobarbital (50 mg/kg). After a cannula had been inserted into the rat’s trachea, the lungs were ventilated with humidified air containing 5% CO2. The ventilator settings included a tidal volume of 1 cm H2O, and a frequency of 60 cycles per minute. After a median sternotomy, the right ventricle was injected with heparin (1 U/g of body weight [BW]), and 10 ml of intracardiac blood were withdrawn. The pulmonary artery was cannulated and perfused with a physiological salt solution. The perfusion rate of the roller pump was set at 8 to 10 ml/min. The PVP (10 cm H2O) and LW, and it was expressed in whole units of g·min−1·cm H2O−1·100 g.10

LW/BW and Wet/Dry Weight Ratios

The right lung was removed after the experiments at the hilar region. The wet LW was then determined, and the LW/BW ratio was calculated. For the dry weight, a part of the right upper lung lobe was dried for 48 h at 60°C in an oven, and the wet/dry (W/D) weight ratio was calculated.

Cell Counts, Protein Concentration, CINC-1, and Tumor Necrosis Factor-α Levels in Bronchoalveolar Lavage Fluid

At the end of the experiment, bronchoalveolar lavage fluid (BALF) was obtained by rinsing the left lung with 2.5 ml of saline twice. The BALF was then centrifuged at 200 g for 10 min. The protein level in the supernatant was measured using a bicinchoninic acid test (Pierce, USA). Tumor necrosis factor (TNF)-α and CINC-1 levels in the BALF were quantified using an enzyme-linked immunosorbent assay kit (R&D Systems Inc., USA). Differential and total cell counts in the BALF were assessed as described previously.11

Malondialdehyde Level and Protein Carbonyl Content in Lung Tissue

The lung tissue was homogenized in a 1.15% KCl aqueous solution. A 100 μl aliquot of the homogenized lung tissue was mixed into a solution of 200 μl of 8.1% thiobarbituric acid and 700 μl of distilled water. The mixture was then boiled for 30 min at 100°C and centrifuged at 3,000 g for 10 min. The malondialdehyde content of the supernatant was measured by absorbance at 532 nm and was expressed as nmol/mg protein. The oxidative damage to the proteins in the lung tissue was assessed by determining the carbonyl group content based on a reaction with dinitrophenyl-hydrazine as previously described.9 The carbonyl content was determined from the absorbance at 370 nm assuming a molar absorption coefficient of 220,000 M−1 and was expressed as the concentration of carbonyl derivatives in the protein (nmol carbonyl/mg protein).9

Western Blot Analysis

Cytoplasmic and nuclear proteins were extracted according to the manufacturer’s instructions (Nuclear/Cytosol Extraction kit; BioVision, Inc., USA) from frozen lung tissue samples and used for Western blot analysis. Protein levels in the extracts were quantified using a bicinchoninic acid protein assay (Pierce). Equal amounts of lung tissue homogenates (30 μg/lane) were electrophoresed on 10 to 12% sodium dodecyl
sulfate–polyacrylamide gels and moved to polyvinylidene fluoro-membranes (Hybond; Amersham Biosciences, USA). The membranes were then incubated in phosphate-buffered saline (PBS) with 0.1% Tween 20 (Sigma-Aldrich, USA) and 5% nonfat milk for 1 h at ambient temperature to prevent nonspecific staining. Primary antibodies against acetylated histone H3 diluted 1:250 (Upstate Biotechnology, USA), heat shock protein (Hsp) 70 diluted 1:500 (Santa Cruz Biotechnology, USA), B-cell lymphoma (Bcl)-2 diluted 1:200 (Santa Cruz Biotechnology), and NF-κB p65 and inhibitor of NF-κB (IκB)-α diluted 1:1,000 (Cell Signaling Technology, USA) were incubated at 4°C overnight. The blots were then washed in PBS with Tween 20 (0.1%) for 10 min and rewashed two more times. The blots were then incubated with horseradish peroxidase–linked anti-rabbit immunoglobulin G (diluted 1:40,000) or anti-mouse immunoglobulin G (diluted 1:50,000) at room temperature for 1 h. The blots were washed three times in PBS with Tween 20 (0.1%) for 10 min. The bands were developed using enhanced chemiluminescence and exposure to x-ray film. Next, the blots were stripped of antibodies and incubated again with an anti-TATA antibody (diluted 1:1,000) for detection of nuclear protein (Abcam, USA). Similarly, anti-β-actin antibody (diluted 1:10,000) was used for detection of cytoplasmic protein (Sigma-Aldrich, USA). The antibody dilutions ensured equivalent loading. The band intensity ratios were calculated.

**Immunohistochemical Studies**

Formalin-fixed, 4-μm paraffin sections were deparaffinized before antigen retrieval. The slides were incubated for 15 min with a solution of 3% H2O2 in methanol to block endogenous peroxidase. The slides were then treated with primary rabbit polyclonal antibody to myeloperoxidase (1:100 dilution; Cell Signaling Technology) and the large activated fragment (17/19 kD) of caspase-3 (1:200 dilution; Cell Signaling Technology). The slides were washed and then incubated for 30 min with rat-specific horseradish peroxidase polymer anti-rabbit antibody (Nichirei Corporation, Japan). Horseradish peroxidase substrate was then added and allowed to react for 3 min, and the sections were then counterstained with hematoxylin.

**Measurement of HO-1 Activity**

Heme oxygenase-1 activity was measured spectrophotometrically in lung tissue by the determination of the rate of appearance of bilirubin as previously described. The level of acetylated histone H3 in lung tissue when compared

**Histological Assessment**

The lung tissue was histologically prepared and stained with hematoxylin and eosin. The numbers of polymorphonuclear neutrophils in the interstitium were counted in 10 high-power fields (×400) and averaged. Two pathologists examined a minimum of 10 randomly selected fields in a masked manner. Lung injury was scored using a four-point scale indicating none (0), mild (1), moderate (2), or severe (3) injury. The slides were examined for neutrophil infiltration in the airspace or vessel wall, and thickening of the alveolar wall. The resulting two scores were added together for the lung injury score.¹²

**Experimental Protocols**

For the VPA series, the rat lungs were randomly allocated to receive PBS (control, n = 6), 300 mg/kg VPA (drug control, n = 6), only I/R, or I/R with different doses of VPA (75 mg, 150 mg, or 300 mg/kg; n = 6 per group). VPA (sodium valproate; Sanofi Aventis Company, USA) was added to the reservoir (containing 20 ml of perfusate). The doses of VPA in this study were chosen according to a previous study.¹³

For the HO-1 activity inhibition series, 36 rats were randomized to receive the vehicle (n = 18, dimethyl sulfoxide; Sigma-Aldrich) or zinc protoporphyrin IX (ZnPP), a specific HO-1 inhibitor (n = 18; dose, 20 mg/kg; Sigma-Aldrich) by intraperitoneal injection 24 h before the experiment. The ZnPP dosage used was chosen based on our previous work showing that ZnPP-suppressed HO-1 activity at 20 mg/kg of BW. The rat lungs were randomly assigned to the control (n = 6), I/R (n = 6), or VPA (300 mg/kg) + I/R (n = 6) groups for the HO-1 inhibitor experiment.

The isolated lungs were allowed to equilibrate for 20 min before starting. The baseline PAP, PVP, weight change, and the initial Kf were measured 60 min later. After all parameters had returned to the baseline state, the lungs were deflated by stopping ventilation and perfusion to cause ischemia. They were maintained in the deflated state for 40 min. Perfusion and ventilation were resumed, and the Kf was measured 60 min later.

**Statistical Analysis**

The data are expressed as means ± SD. The nature of hypothesis testing was two tailed. For comparisons of LW gain and PAP between groups during 60 min of observation, a two-way ANOVA for repeated measurements was used followed by the post hoc Bonferroni test. The comparisons among the groups were performed by using one-way ANOVA followed by a post hoc Bonferroni test. Comparisons for Kf within each group were performed using paired Student t tests. There were no missing data points in this experiment. The sample sizes in the current study were justified based on our previous works. The six animals in each group were appropriate to avoid intravariability in the experimental group. Significance was set at the P value less than 0.05. GraphPad Prism 6 (GraphPad Software, USA) was used to perform the statistical calculations.

**Results**

**Effect of VPA on Acetylated Histone H3 Level**

The acetylation of lys9 in histone H3, an index of HDAC inhibition, was measured, and VPA significantly increased the level of acetylated histone H3 in lung tissue when compared
with the I/R group (fig. 1). The HDAC inhibition was dose dependent. This result indicates that the dose of VPA in this study was sufficient to increase acetylation of histone protein.

**Effect of VPA on Lung Edema**

Ischemia–reperfusion significantly increased LW gain (fig. 2A). This increase in the LW gain was attenuated by the VPA treatment in a dose-dependent manner. Moreover, the addition of ZnPP blocked the protective effect of VPA. I/R significantly increased $K_r$, LW/BW and W/D weight ratios, and protein levels in the BALF ($P < 0.05$; fig. 2, B–E); VPA treatment significantly reduced these increases in a dose-dependent manner. However, the addition of ZnPP blocked the protective effect of VPA.

**Effect of VPA on PAP**

In the control group, the PAP had almost no change during the 100-min observation interval. In the I/R group, the PAP increased and then decreased to its lowest point 20 min after reperfusion. At 60 min after reperfusion, the PAP in the I/R group remained significantly higher than at baseline and that of the control group. Treatment with VPA significantly diminished the increase in this late stage of PAP increase in a dose-dependent manner. The protective effect of VPA was significantly blocked when ZnPP was added ($P < 0.05$; fig. 3).

**Effect of VPA on HO-1 Activity in Lung Tissue**

Ischemia–reperfusion significantly increased HO-1 activity when compared with the control group ($P < 0.05$; fig. 4). Furthermore, VPA (300 mg/kg) treatment significantly enhanced lung HO-1 activity in comparison with the I/R group. In addition, ZnPP treatment reduced HO-1 activity significantly in the I/R plus VPA group ($P < 0.001$; fig. 4).

**Effect of VPA on TNF-α and CINC-1 Levels in BALF**

The TNF-α and CINC-1 levels were significantly increased in the BALF of the I/R group in comparison with those in the control group (fig. 5). VPA (300 mg/kg) significantly inhibited the production of TNF-α and CINC-1 in the I/R group. ZnPP treatment abolished the protective effects of VPA.

**Effect of VPA on Malondialdehyde Level, Carbonyl Content, and Myeloperoxidase-positive Cells in Lung Tissue**

In comparison to the control group, the I/R group had significantly increased malondialdehyde levels, carbonyl contents, and numbers of myeloperoxidase-positive cells in the lung tissue ($P < 0.001$; fig. 6, A–C). Treatment with VPA (300 mg/kg) significantly attenuated these increases ($P < 0.001$). However, the protective effects of VPA were abolished by treatment with ZnPP.

**Effect of VPA on Histopathology and Lung Injury Score**

The lung histology disclosed obvious interalveolar septum thickening and increased inflammatory cell infiltration in the I/R group compared with the control group (fig. 7A). The VPA-treated group showed decreased neutrophil infiltration (fig. 7B), lung injury scores (fig. 7C), and neutrophil migration into the alveolar spaces (fig. 7D). In contrast, the protective effect of VPA was abolished by pretreatment with ZnPP.

**Effects of VPA on Hsp70, Bcl-2, and Caspase-3 Protein Expression**

Hsp70 and Bcl-2 protein contents in lung tissue were substantially decreased in the I/R groups than in the control groups. However, both proteins were significantly increased upon VPA treatment. The protective effect of VPA was abolished by treatment with ZnPP (fig. 8, A and B). The intensity of activated caspase-3-immunolabeled cells was significantly greater in the I/R group than in the control group. VPA treatment significantly decreased immunolabeled cells, but the protective effect was reduced by the addition of ZnPP (fig. 8C).

**Effect of VPA on NF-κB Signaling Pathway**

The nuclear level of NF-κB p65 was increased after I/R injury (fig. 9A), whereas the cytoplasmic level of IkB-α was decreased significantly in the I/R group compared with the control group (fig. 9B). VPA treatment restored suppressed IkB-α levels and reduced nuclear NF-κB p65 levels. Treatment with ZnPP counteracted the protective effect of VPA (fig. 9).

**Discussion**

The current study demonstrated that acute lung injury induced by I/R significantly increased lung edema, PAP, neutrophil infiltration, inflammatory cytokine production, oxidative stress, apoptosis, IkB-α degradation, nuclear

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Fig. 1. Effect of valproic acid (VPA) on acetylated histone H3 (Ac-H3) expression in lung tissue. VPA up-regulated Ac-H3 expression in a dose-dependent manner in ischemia–reperfusion (I/R) lung injury. TATA served as loading controls for nuclear proteins. A representative blot is shown. Data are expressed as mean ± SD (n = 3 per group). ***P < 0.001 compared with the control group; +++P < 0.001 compared with the I/R–vehicle group. *P < 0.05; **P < 0.01; +P < 0.01.
translocation of NF-κB, and tissue injury. These effects were significantly attenuated by pretreatment with VPA. However, the protective effect of VPA was reduced by the HO-1 activity inhibitor, ZnPP. This indicates that VPA exerts its antiinflammatory effects partly through enhancing HO-1 activity. Our results suggest an important role for HDAC in I/R-induced lung injury and also that HO-1 could contribute to the protection provided by VPA.

Fig. 2. Effect of valproic acid (VPA) and zinc protoporphyrin IX (ZnPP) on pulmonary edema. Lung weight gain (A), Kf (B), lung weight/body weight (LW/BW) (C), and wet/dry (W/D) weight ratios (D), and protein concentration in bronchoalveolar lavage fluid (BALF) (E) increased significantly in the ischemia–reperfusion (I/R) group. The increase in these parameters was significantly attenuated by treatment with VPA. The protective effect of VPA was abrogated by ZnPP treatment. Data are expressed as mean ± SD (n = 6 per group). **P < 0.01, ***P < 0.001 compared with the control group; +P < 0.05, ++P < 0.01, +++P < 0.001 compared with the I/R–vehicle group; #P < 0.05, ###P < 0.001 compared with the I/R + VPA 300 group.
HDAC inhibitors have emerged as potent antiinflammatory therapeutic agents in a variety of diseases based on their ability to increase histone acetylation, alter the transcription of associated genes, and provide tissue protection. After I/R injury, there was a significant decrease in the histone H3 acetylation in the lung tissue, similar to results from a previous study using a rat model of permanent ischemic stroke. The reduction in acetylated histone could be related to the suppression of histone acetyltransferase and/or activation of HDAC after an I/R insult. I/R-induced loss of histone acetylation was prevented by VPA treatment. These results indicate that HDAC activity was inhibited in the rat lungs by VPA treatment under the experimental conditions.

Neutrophil sequestration and transmigration in the lung interstitium and alveolar space are the primary characteristics of acute respiratory distress syndrome. The number of infiltrating neutrophils was significantly associated with the
severity of tissue damage after I/R injury. Several studies also showed that suppression of neutrophil infiltration improved reperfusion injury and revealed that I/R lung injury was dependent on neutrophil recruitment. The results demonstrated that I/R increased neutrophil infiltration in lung tissue, as evidenced by both increasing myeloperoxidase-positive cells and the number of neutrophils, both of which were attenuated by VPA treatment. This decreased the interaction between neutrophils and the endothelium and reduced the release of proinflammatory cytokines, generation of reactive oxygen species, and free radicals by activated neutrophils. Moreover, this VPA attenuation decreased pulmonary edema as indicated by the reduced $K_F$, lower W/D and LW/BW ratios, and decreasing the protein concentration in the BALF. These results agree with those of other investigations showing that HDACIs have the ability to attenuate vascular permeability and neutrophil infiltration in various models of acute lung injury.

Investigators of clinical and experimental studies have suggested that the oxidative stress induced by reactive oxygen species plays an important role in I/R-induced lung injury. In addition, neutrophil-derived oxygen radicals injure vascular endothelial cells both in vitro and in vivo, disrupt the functional integrity of the microvasculature, and increase fluid and protein flow from the vascular space into the interstitium. Our results demonstrated that VPA prevented the protein carbonylation and peroxidation of membrane lipids during lung I/R. Similarly, other studies showed that VPA treatment also improved pulmonary oxidative damage and exerted antioxidative effects on the retina after I/R injury.

The significance of cytokines in the pathogenesis of acute lung injury has been validated by the widespread use of anti-cytokine monoclonal antibodies. Our experiment showed that the expression of inflammatory mediators such as TNF-α and CINC-1 in BALF were up-regulated after I/R-induced lung injury. In parallel with this result, activated caspase-3-immunolabeled cells also increased accompanying the decreased expression of Hsp70 and Bcl-2 proteins after...
lung injury. Furthermore, VPA significantly attenuated the increased levels of inflammatory mediators and apoptotic cell death. Indeed, HDACIs reduce proinflammatory cytokine production as well as inhibit cytokine effects.1,3 Our data were also comparable to that of several investigations showing that the HDACI-induced neuroprotection is due to its antiapoptotic effects. In the rodent model of middle cerebral artery occlusion, HDACIs suppressed ischemia-induced neuronal caspase-3 activation and increased antiapoptotic molecule expression of Hsp70 and Bcl-2, both of which protected against ischemic neuronal death.14,21 However, further studies are required to examine the VPA-associated epigenetic control mechanisms of proinflammatory cytokines and apoptotic processes.

Nuclear factor-κB is essential for activating the transcription of a proinflammatory cascade of cytokines and chemokines to induce early inflammatory responses. NF-κB-modulated gene expression is also regulated by posttranscriptional modifications, such as phosphorylation and acetylation, which can be altered upon stimulation.3 NF-κB activation is tightly regulated by its endogenous inhibitor, IκB, which complexed with NF-κB in the cytoplasm. Previous studies showed that I/R lung injury degraded IκB and induced NF-κB activation.7,12 Our results demonstrated that VPA prevented nuclear translocation of NF-κB and inhibited the degradation of IκB as well as NF-κB-regulated gene expression. Indeed, HDACIs induce hyperacetylation and repress NF-κB signaling in vitro and in vivo.5,5,22 The reduction of NF-κB translocation in macrophages within the lamina propria was beneficial in patients with ulcerative colitis treated with HDACI.23 However, other investigators suggest that HDACIs enhanced NF-κB-dependent gene expression.22 This indicates that the

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Fig. 7. Effect of valproic acid (VPA) and zinc protoporphyrin IX (ZnPP) on inflammatory cell infiltration and the histological appearance of lung tissue. As shown by a representative micrograph of lung tissue (×400 magnification) (A), infiltrating neutrophils and sepal widening were increased in the ischemia–reperfusion (I/R) group. VPA treatment improved the histopathological changes, but the improvement was abolished by ZnPP treatment. The lung injury scores (B), the numbers of neutrophils per high power field (×400 magnification) (C), and the percentage of neutrophils in bronchoalveolar lavage fluid (BALF) (D) were significantly higher in the I/R group than in the control group. VPA treatment significantly decreased the rise, but these improvements were abolished by ZnPP treatment. Data are expressed as mean ± SD (n = 6 per group). **P < 0.01, ***P < 0.001 compared with the control group; +++P < 0.001 compared with the I/R–vehicle group; #P < 0.05, ###P < 0.001 compared with the I/R + VPA group. *P < 0.05; +P < 0.05.
effects of both histone acetyltransferases and HDAC inhibition depend on acetylation/deacetylation of NF-κB at different lysine residues and the cell type. The precise molecular mechanisms by which HDAC inhibition exerts its effects in these models remain to be elucidated.

The protective effects of VPA involve multiple mechanisms. HDACIs not only directly modulate acetylation of nuclear histones but other nonhistone proteins as well, including regulatory proteins involved in the gene expression, cell death (apoptosis), autophagy, cell cycle progression, redox pathways, DNA repair, cell migration, and angiogenesis. Furthermore, other mechanisms still need clarification. Our results showed that VPA treatment enhanced HO-1 activity in I/R lung injury. HO-1 is the inducible form of HO and the rate-limiting enzyme in heme degradation. Many studies revealed that HO-1 has a much larger role in tissue damage and protection, including antioxidant, antiapoptotic, and antiinflammatory actions. In the current study and previous experiments, I/R alone resulted in increased HO-1 enzyme activity. VPA further enhanced HO-1 activity and most importantly, it attenuated I/R-induced lung injury. In contrast, HO-1 inhibitor reversed the protective effect of VPA. Our results agree with those of previous investigations that increased HO-1 activity due to ischemic postconditioning, administration of HO-1 adenovirus, and hypercapnic acidosis reduces lung reperfusion injury. Furthermore, two recent studies demonstrated that HDACIs significantly increased HO-1 expression in the brain, cardiomyocytes, and hearts subjected to ischemic injury. In addition, HO-1-deficient mice were unprotected by pharmacologic inhibition of HDAC after cerebral ischemia.
Valproic acid is widely used for epilepsy therapy, bipolar disorder, and some painful neuropathies. It is well known to inhibit HDAC classes I and II, but not class III. In addition, different HDACIs have particular actions on individual acetylation sites, which could provide unique therapeutic effects. Currently, 18 different HDACs have been identified in mammals. A recent study showed that HDAC 7 represented a potential inflammatory disease target to enhance the Toll-like receptor-4–dependent inflammatory response. However, the roles of the different HDAC isoforms and HDACIs in I/R-induced lung injury remain poorly understood. Therefore, we believe that further investigations will focus on identifying the functions of the different HDAC enzymes and investigating isoform-specific HDACIs in animal models of acute respiratory distress syndrome.

In summary, we demonstrated that VPA attenuated lung I/R injury by decreasing lung edema, production of inflammatory cytokines, reactive oxygen species, NF-κB signaling, and apoptosis, whereas it enhanced HO-1 activity. The protective effect of VPA was mitigated by the presence of ZnPP, a HO-1 activity inhibitor. These data support the notion that VPA-induced HO-1 expression is responsible, at least in part, for the beneficial effects of VPA. Our results suggest that VPA provides effective treatments for I/R-induced acute lung injury. Further studies should provide greater knowledge for a better understanding of the protective mechanism of individual HDAC isoform inhibitors.

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Competing Interests

The authors declare no competing interests.

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References

lipopolysaccharide-induced acute lung injury in mice. Respir Res 2010; 11:33