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FULL PAPER

Pharmacology

Regulatory effect of *Panax notoginseng* saponins on the oxidative stress and histone acetylation induced by Porcine circovirus type 2 infection *in vitro* and in mice

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ABSTRACT.

Porcine circovirus type 2 (PCV2) exists widely in swine populations worldwide, and healthy PCV2 virus carriers have enhanced the severity of the infection, which is becoming more difficult to control. This study investigated the regulatory effect of *Panax notoginseng* saponins (PNS) on the oxidative stress and histone acetylation modification induced by PCV2 *in vitro* and in mice. *In vitro*, PNS significantly increased the scavenging capacities of superoxide anion radicals ($O_2^{\cdot-}$) and hydroxyl radicals ($\cdot OH$) and reduced the content of hydrogen peroxide (H_2O_2) induced by PCV2 in porcine alveolar macrophages (3D4/2). In addition, PNS decreased the protein expression level of histone H4 acetylation (Ac-H4) by increasing the activity of histone deacetylase (HDAC) in PCV2-infected 3D4/2 cells. *In vivo*, PNS enhanced the scavenging capacities of $\cdot OH$ and $O_2^{\cdot-}$ and reduced the content of H_2O_2 in the spleens of PCV2-infected mice. PNS also reduced the protein expression level of histone H3 acetylation (Ac-H3) by reducing the activity of histone acetylase (HAT) and increasing the activity of histone deacetylase (HDAC) in the spleens of PCV2-infected mice. PCV2 infection activated oxidative stress and histone acetylation *in vitro* and in mice, but PNS ameliorated this oxidative stress. The research can provide experimental basis for exploring the antioxidant effect and the regulation of histone acetylation of PNS on PCV2-infected 3D4/2 cells and mice *in vitro* and *in vivo*, and provide new ideas for the treatment of PCV2 infection.

KEY WORDS: antioxidation, histone acetylation, porcine circovirus type 2, *panaxnotoginseng* saponins

INTRODUCTION

Porcine circovirus type 2 (PCV2), a single-strand circular DNA virus, is a major pathogen of postweaning multisystemic wasting syndrome (PMWS) [22]. Oxidative stress reflects the imbalance between oxidation and antioxidation, which leads to oxidative damage of lipids, proteins, DNA and other macromolecules [20, 21]. In swine, PCV2 infection mainly triggers the immune system. It also similarly reduces the immunity of organisms and their immune responses to the pathogen, which in turn increases the virulence of the infection of swine or the secondary infection of other pathogenic microorganisms. In addition, PCV2-induced oxidative stress and immunosuppression can promote the replication of viruses, exacerbate the symptoms of the disease and ultimately cause tremendous economic losses to the swine industry [12, 16, 29]. Therefore, it is important for us to find a way to prevent PCV2 infection.

Saponins are deemed to have anti-inflammatory, antioxidant, antiviral, anti-aging and immune strengthen and other functions [7, 8, 24, 31, 32]. Ginsenosides (GSRs) protect cells from damage by regulating the balance of histone acetylase and histone deacetylase. GSRs combine to restore the balance between Ac-H3 and histone deacetylase 2 (HDAC2) and improve the survival rate of brain-derived neurotrophic factor (BDNF) and neurons, thus promoting the functional capacity of neurons [23]. In addition, GSRs inhibit the proliferation of K562 and KG1- α cells by modulating the expression and activity of HDAC, increasing histone acetylation and modulating key proteins in downstream signaling pathways [13]. *Panax notoginseng* saponins (PNS) are the main active ingredients isolated from *Panax notoginseng* with various functions, including antiviral, anti-inflammation, antioxidation and immunity improvement [15]. Among these functions, their antioxidant effects are receiving the most attention. PNS plays role in protecting microvascular endothelial cells in the brain from oxidative damage by activating the PI3K/Akt/Nrf2 signaling pathway [5]. Saponin Rh4, obtained from *Panax notoginseng*, triggers apoptosis and autophagy by activating the JNK/p53 pathway in colorectal cancer cells [27]. Thus, histone acetylation and histone deacetylation may regulate gene transcription by affecting DNA-interacting proteins, and this state could be regulated by oxidative stress in cells [6].

Despite previous studies being conducted on the antioxidant effect, there is still a lack of literature on the epigenetic changes of PNS both *in vitro* and *in vivo*. Therefore, we selected 3D4/2 cells and mice spleens to explore the regulation of PNS on oxidative stress and histone acetylation caused by PCV2 infection *in vitro* and in mice.

MATERIALS AND METHODS

Materials

PNS was extracted by the alcohol extraction method by the Department of Pharmacology, College of Animal Science and Technology, Guangxi University. The purity of the PNS was determined to be 70.52% after being subjected to a D101 macroporous resin. PNS was dissolved in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS) and sterilized by filtration through a 0.22 μ m membrane to a concentration of 200 μ g/ml. Vitamin c (V_C) was purchased from Sigma (California, U.S.A.), and V_C solution was prepared in a manner similar to that of PNS. PCV2 was isolated and stored by the Animal Disease Diagnosis and Immunology Laboratory of Nanjing Agricultural University. The virus titer of PCV2 obtained by proliferation on PK-15 cells was 10^4 median tissue culture infective dose (TCID₅₀)/0.1 ml. The PCV2-SH strain (GenBank accession no.

AY686763) was obtained from the Key Laboratory of Animal Disease Diagnosis and Immunology and Ministry of Agriculture, College of Veterinary Medicine, Nanjing Agricultural University [4, 11]. The PCV2-SH was isolated from pigs with Postweaning Multisystemic Wasting Syndrome (PMWS) in Shanghai, China in 2004, and has been shown to induce an apparent disease [25]. The Inhibition and Produce Superoxide Anion Assay kit, Hydroxyl Free Radical assay kit and Hydrogen Peroxide assay kit were purchased from Nanjing Jiancheng Bioengineering Institute. Histone acetylase (HAT) ELISA kit for mice and histone deacetylase (HDAC) ELISA kit for mice, histone acetylase (HAT) ELISA kit for porcine and histone deacetylase (HDAC) ELISA kit for porcine were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China). Histone acetylation Ac-H3 and Ac-H4 primary antibodies were purchased from Millipore, (Massachusetts, U.S.A.). β -actin antibody and anti-rabbit IgG horseradish peroxidase (HRP)-linked antibody were purchased from CST (Massachusetts, U.S.A.). Bicinchoninic Acid Assay (BCA) protein assay kit was purchased from Beijing ComWin Biotech Co., Ltd. (Beijing, China).

Cell culture

3D4/2 cells were provided by the Department of Preventive Veterinary Medicine, College of Animal Science and Technology, Guangxi University (ATCC: CRL-2845). Some previous studies have shown that PCV2 could infect porcine alveolar macrophage 3D4/2 cells (ATTC: CRL 2845) [3, 9, 14]. The 3D4/2 cells were recovered and passaged with 10%-FBS-RPMI 1640 medium and cultured at 37°C in a 5% CO₂ incubator. After the cells reached a certain number, the cell concentration was adjusted for trypan blue staining assay.

Experimental animals

The protocol and procedures employed were ethically reviewed and approved by the ethical committee of Guangxi University (animal study approval number: SCXK Gui 2019-0003). The experiments were performed in accordance with Guangxi University guidelines and regulations for the care and use of laboratory animals. One hundred and twenty-six SPF Kunming mice (one-half male and one-half female, weighing 20 ± 2 g) were provided by the Experimental Animal Center of Guangxi Medical University. All mice were divided into seven groups, with 18 mice in each group (half male and half female). They were maintained in sterilized squirrel cages, with regular feeding and a constant temperature of $22 \pm 2^\circ\text{C}$ in a 12 hr of light and 12 hr of dark photoperiod environment. To reduce stress, the purchased mice were allowed to adapt fully to the environment with the ability to eat and drink freely

for five days. The mice were fasted for 12 hr and water deprived for 6 hr before the experiment.

PCV2 infection and PNS treatment of the 3D4/2 cells

3D4/2 cells were plated in 6-well plates at 2 ml per well and cultured at 37°C in a humidified incubator with 5% CO₂ for 2 hr. After the cells adhered to the plate walls, the supernatant was discarded, and the cells were washed with 0.1 M phosphate buffer saline (PBS) three times. The cells were allocated into five groups: A (Control group), B (10⁻¹ PCV2 group), C (PNS group), D (PCV2+PNS group) and E (PNS+PCV2 group). Groups A, B, C, and D were treated with freshly prepared culture solution, and group E was treated with PNS solution. After culturing at 37°C in a incubator with 5% CO₂ for 12 hr, the cells were washed with 0.1 M PBS 3 times. Groups A and C were supplemented with 1.0 ml of culture medium, while groups B, D and E were added with 1 ml of 10⁻¹ PCV2 solution. The cells were cultured for another 2 hr and gently shaken every 15 min. The supernatant was discarded after 2 hr, and the cells were washed twice with 0.01 M PBS, RPMI 1640 medium was added to groups A, B and E, and PNS (200 µg/ml) was added to groups C and D. Then, the cells were cultured for another 12 hr.

After the cells cultured in the 6-well plate were washed three times with sterilized physiological saline, the cells were scraped off the walls with a cell scraper. The cells were sonicated in an ice water bath for 1 min and centrifuged at 12,000 rpm for 15 min at 4°C. Then, the supernatant was collected and stored at -80°C. The supernatant was used to detect the content of H₂O₂, the scavenging capacities of [•]OH and O₂^{•-}, and the activities of HAT and HDAC.

In addition, the treated cells cultured in the 6-well plates were washed with 0.01 M PBS three times, and then, 1 ml of precooled 0.01 M PBS was added. The cells were scraped off the cell walls with a cell scraper, placed into a 1.5 ml Eppendorf tube, and centrifuged at 1500 rpm for 10 min. Then, the supernatant was discarded, and the process was repeated three times. The cell precipitates were rapidly frozen in liquid nitrogen or -80°C for the extraction of histones and Western blotting experiments.

PCV2 infection and PNS treatment of the mice

One hundred and twenty-six mice were randomly allocated to one of seven groups (A, B, C, D, E, F, and G groups), with 9 females and 9 males in each group. After the start of the experiment, on days 1, 2, and 3, the mice in groups B, C, D, E, and F were coinfectd with 1.0 ml of PCV2 stock by intraperitoneal injection, oral administration and intranasal drip, while those in groups A and G were administered an equivalent dose of saline.

Then, 4, 5, and 6 days after the start of the experiment, the mice in groups A and B were

administered normal saline by intraperitoneal injection, while PNS was administered by intraperitoneal injection to the mice in groups C (50 mg/kg·bodyweight (BW)), D (100 mg/kg·BW), E (200 mg/kg·BW), and G (200 mg/kg·BW). The mice in group F were intraperitoneally injected with Vc (100 mg/kg·BW). All mice were deprived of food and water for 12 hr and 6 hr, respectively, prior to PCV2 inoculation. Thereafter, the mice were raised routinely under the conditions described above, as shown in the test flow diagram in **Fig. 1**.

Seven days after infection, the mice from each treatment group were sacrificed by CO₂ asphyxia, and the spleen of each mouse was collected. The spleens were washed with sterile physiological saline, blotted with filter and weighed immediately. 9 ml/g spleen weight of sterile physiological saline was added to prepare a 10% tissue homogenate. The homogenate was centrifuged at 5000 rpm for 10 min, and the supernatant was stored at -80°C for the determination of H₂O₂ content, the scavenging capacities of [•]OH and O₂^{•-}, and the activities of HAT and HDAC.

Finally, the remaining spleen tissue was placed in a 1.5 ml centrifuge tube, and 1.0 ml of precooled PBS was added and centrifuged at 2000 rpm for 5 min at 4 °C, and then, the supernatant was discarded. The blood on the surface of spleen was washed twice to extract histone for Western blotting experiment.

Chemiluminescence assay determination of O₂^{•-} and [•]OH inhibition and H₂O₂ content

The anti-superoxide anion activity was detected according to the xanthine and xanthine oxidase reaction system method, the inhibition of [•]OH was determined according to the Fenton reaction, and the H₂O₂ level was determined according to the molybdcic acid reaction. Each operation was carried out in accordance with the instructions in the Inhibition and Produce Superoxide Anion Assay kit, Hydroxyl Free Radical assay kit and Hydrogen Peroxide assay kit, respectively.

HAT and HDAC analysis

The activities of HAT and HDAC in the 3D4/2 cells and mouse spleens were detected by ELISA kits. All operations were performed in accordance with the kits' instructions.

Extraction and quantification of histones

The concentrations of histones in the 3D4/2 cells and spleens were measured by the BCA method. The protein standard, bovine serum albumin (BSA), was diluted with PBS to a final concentration of 0 µg/µl, 0.0625 µg/µl, 0.125 µg/µl, 0.25 µg/µl, 0.5 µg/µl, 1.0 µg/µl, and 2.0 µg/µl. A total of 8.0 µl of each histone sample was extracted and diluted 10 times with PBS. Solutions A and B in the BCA kit were mixed at a ratio of 50:1. The 25 µl per well of diluted BSA standard and 25 µl per well of protein samples

was added to a 96-well plate, with three replicates per sample. Two hundred microliters of premixed solution A and B were added to the sample well or standard well, mixed well, covered with a 96-well plate cover, and incubated at 37°C for 30 min. The light absorption value of each sample and the standard sample at a wavelength of 562 nm was measured by a multifunctional microplate micrograph and recorded. The standard curve was drawn, and the protein concentration of samples were calculated according to the standard curve.

Western blotting

The total protein concentration of the samples were determined with a BCA protein assay kit. After adjusting the protein concentration of each sample to a uniform standard, an appropriate volume of 5× loading buffer was added and mixed, and then, the sample was subject to heat denaturing at 100°C. A protein amount equivalent to 20 µg of histone per well was loaded for separation by SDS-PAGE, and then the protein was transferred to a membrane by the constant current on a semi-dry film transfer apparatus under the following conditions: current = membrane area × number of membranes×1.5 mA. After protein transfer, the PVDF membranes were blocked with 5% skim milk overnight at 4°C or for 1 h at 37°C. Each blocked PVDF membrane was washed three times with Tris Buffered Saline Tween (TBST) for 10 min each time, and then put into diluted primary antibodies (β-actin, Ac-H3 or Ac-H4) for incubation at 4°C overnight. The PVDF membranes with the primary antibody were washed three times with TBST, loaded into an antibody incubation cassette with diluted HRP-labelled anti-rabbit IgG secondary antibody and incubated at 37°C for 1 hr. The protein bands on the membranes were visualized using a chemiluminescence imager in the Chemi Doc MP protein-imaging system. The grey value of the protein bands was analysed using Quantity One analysis software. With β-actin serving as an internal reference, the relative grey values of the protein bands were compared. High relative grey values indicated high levels of Ac-H3 or Ac-H4.

Statistical analysis

The experimental data were analysed by one-way analysis of variance (ANOVA) using SPSS 21.0 statistical software, and the Duncan posthoc test was used to analyse the difference among treatment groups for which significance ($P < 0.05$) was determined. The results are expressed as the means ± SD. All values of $P < 0.05$ were regarded as statistically significant. After processing the data with SPSS, then make a small picture in Microsoft excel, and finally use Photoshopcs6 to combine the small pictures into a large picture.

RESULTS

Effects of PNS on 3D4/2 cells infected with PCV2 in vitro

Effect of PNS on the antioxidant capacity of PCV2-infected 3D4/2 cells

The scavenging capacities of $O_2^{\cdot-}$ (**Fig. 2A**) and $\cdot OH$ (**Fig. 2B**) in the cells of the PCV2 group were significantly lower than that in the cell control group ($P < 0.05$). The content of H_2O_2 in the PCV2 group was slightly higher than it was in the cell control group, with no significant difference ($P > 0.05$) (**Fig. 2C**).

After the PCV2-infected cells were treated with 200 $\mu g/ml$ PNS for 12 hr, the scavenging capacities of $O_2^{\cdot-}$ (**Fig. 2A**) and $\cdot OH$ (**Fig. 2B**) were upregulated, and the content of H_2O_2 was dramatically downregulated compared with the PCV2 group ($P < 0.05$, $P < 0.01$) (**Fig. 2C**). The scavenging capacities of $O_2^{\cdot-}$ (**Fig. 2A**) and $\cdot OH$ (**Fig. 2B**) in the cells treated with PNS and then infected with PCV2 were significantly higher than those in the PCV2 group ($P < 0.05$, $P < 0.01$).

Effect of PNS on the activities of acetylation-related enzymes in PCV2-infected 3D4/2 cells

The activity of HAT in the PCV2-infected 3D4/2 cells was significantly increased compared with that in the cells of the control group ($P < 0.05$) (**Fig. 3A**). The activity of HDAC in the PCV2-infected 3D4/2 cells was significantly lower than that in the cells of the control group ($P < 0.05$). When PNS treated 3D4/2 cells infected by PCV2 for 12 hr, HDAC activity was significantly increased ($P < 0.05$), while HAT activity was lower than that of the PCV2 group, with no significant difference ($P > 0.05$) (**Fig. 3B**).

Effect of PNS on the protein expression levels of Ac-H3 and Ac-H4 in PCV2-infected 3D4/2 cells

The protein expression levels of Ac-H3 and Ac-H4 are shown in **Fig. 4**. PCV2 increased the protein expression level of Ac-H3 in 3D4/2 cells, while PNS decreased the protein expression level of Ac-H3 in PCV2-infected 3D4/2 cells, with no significant difference ($P > 0.05$). The protein expression level of Ac-H4 was significantly increased after the cells were infected with PCV2 ($P < 0.05$) and then was significantly reduced after the PCV2-infected cells were treated with PNS (200 $\mu g/ml$) for 12 hr ($P < 0.05$).

Effects of PNS on the spleens of PCV2-infected mice

Effect of PNS on the antioxidation ability in the spleens of PCV2-infected mice

The results of anti-free radical activity and H_2O_2 content are shown in **Fig. 5**. PCV2 significantly

reduced the scavenging capacities of $\cdot\text{OH}$ (**Fig. 5A**) and $\text{O}_2^{\cdot-}$ (**Fig. 5B**) ($P < 0.05$) and increased the content of H_2O_2 in the spleens compared with the control group ($P > 0.05$) (**Fig. 5C**). The medium and high doses of PNS and the V_C treatment significantly increased the scavenging capacities of $\cdot\text{OH}$ (**Fig. 5A**) and $\text{O}_2^{\cdot-}$ (**Fig. 5B**) in the PCV2-infected mice ($P < 0.05$). After being infected with PCV2, the mice in the PNS groups were intraperitoneally injected with different concentrations of PNS, and the content of H_2O_2 in the spleens of the infected mice was reduced, with no significant difference between the mice treated with or without PNS ($P > 0.05$).

Effect of PNS on the activities of acetylation-related enzymes in the spleens of PCV2-infected mice

The results of the activities of HAT and HDAC in the spleens of the mice are shown in **Fig. 6**. Compared to the control group, the activity of HAT was significantly increased after PCV2 infection ($P < 0.05$). After the mice were infected with PCV2, the activity of HAT decreased despite the mice being treated with PNS or V_C , except in the mice administered the low dose of PNS, with no significant difference ($P > 0.05$). After the mice were infected with PCV2, the activity of HDAC in their spleens was significantly decreased compared to the activity in the spleens in the control group ($P < 0.05$). Different concentrations of PNS increased the activity of HDAC in the spleens of PCV2-infected mice, with no significant difference ($P > 0.05$). Additionally, there was no significant difference in the activity of HDAC between the V_C group and the PCV2 group ($P > 0.05$).

Effect of PNS on the protein expression levels of Ac-H3 and Ac-H4 in the spleens of PCV2-infected mice

PCV2 significantly increased the protein expression level of Ac-H3 in the mice compared to the level in the control group ($P < 0.05$) (**Fig. 7**). Treatment with any of the three doses of PNS or with V_C decreased the protein expression level of Ac-H3 in mice infected with PCV2, but only the 100 mg/kg·BW treatment had a significant effect ($P < 0.05$). PCV2 increased the protein expression level of Ac-H4, with no significant difference ($P > 0.05$). V_C and different doses of PNS could reduce the protein expression level of Ac-H4 in the spleens of PCV2-infected mice, with no significant difference ($P > 0.05$).

DISCUSSION

Given the great loss in the livestock industry caused by PCV2 infection, preventing infection has received increasing attention. In this study, we studied the effect of a natural Chinese herbal medicine,

Panax notoginseng, in which PNS is the main active ingredient, on antioxidant capacity and epigenetic regulation *in vitro* and in mice.

In this study, the scavenging capacities of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ were significantly reduced in PCV2-infected 3D4/2 cells *in vitro*. The increase of H_2O_2 content in PCV2-infected 3D4/2 cells was not significant. These findings indicate that the oxidative damage caused by PCV2 infection may not depend on an increase in H_2O_2 content. The other reason may be related to the activation of the intracellular antioxidant enzyme system after PCV2 induced the degradation of the generated hydrogen peroxide. PNS treatment did not change the scavenging capacities of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ or H_2O_2 content *in vitro* and in mice not infected with PCV2. However, PNS significantly increased the scavenging capacities of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ in PCV2-infected 3D4/2 cells compared with the PCV2 group, indicating that PNS can improve the antioxidant capacity of PCV2-infected 3D4/2 cells. The effects of PCV2-infected mice and PNS on the antioxidant activity of PCV2-infected mice were similar to that *in vitro*. First, the scavenging capacities of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ in PCV2-infected mice were significantly reduced. However, similar to *in vitro* experiments, there was no significant increase in H_2O_2 content in the spleens of PCV2-infected mice. Second, PNS increased the scavenging capacities of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ and reduced the content of H_2O_2 in PCV2-infected 3D4/2 cells and mice spleens. These results are consistent with those of previous studies showing that *Sapindus mukorossi* Gaertn, ginsenoside Rg3 and gypenoside L (Gyp-L) are effective in clearing cellular reactive oxygen species and exhibit strong antioxidant activity [19, 2]. It is worth noting that PNS has a dose-dependent effect on the antioxidant activity of PCV2-infected mice. The middle and high doses of PNS can significantly enhance the scavenging abilities of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ in the spleens of PCV2-infected mice. Nevertheless, all these treatments had no significant effect on the content of H_2O_2 , which provided evidence that the oxidative damage caused by PCV2 infection may not depend on the increase of H_2O_2 content. Thus, PNS was likely able to increase the scavenging capacities of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ in PCV2-infected 3D4/2 cells and mice, which in turn increased antioxidant activity.

The results from our previous studies showed that *Sophora subprostrate* polysaccharides antagonize the inflammatory response of PCV2-infected 3D4/2 cells by participating in the mechanism of histone acetylation and the NF- κ B signaling pathway [28]. *Sargassum* polysaccharides may be able to inhibit the inflammatory response by maintaining a balance between the activities of HAT and HDAC [1]. As a major regulator of protein acetylation, HDAC is a necessary transcriptional and epigenetic regulator. In the present study, we also found that the effect of PNS on antioxidant activities and

epigenetic changes to be similar to those of the *Sophora subprostrate* polysaccharides. PCV2 infection significantly increased HAT activities and decreased HDAC activities *in vitro* and in mice, indicating that PCV2 infection not only caused oxidative stress but also changed the balance between acetylation and deacetylation of histones to acetylation. These results are consistent with the results of Wei et al., who showed that curcumin inhibited HBV gene replication by downregulating the acetylation of cccDNA-binding histones and therefore may be developed as a cccDNA-targeting antiviral medicine for treating hepatitis B [26].

The imbalance of histone acetylation is known to be closely related to many diseases, such as heart disease, cancer and viral infections [18,30]. Relevant studies have shown that viruses, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and influenza A virus, can disrupt the function of HDAC through various mechanisms [17]. Herpes simplex virus (HSV-1)-infected human foreskin fibroblast cells (HFFs) have an increased Ac-H3 level, which is an indicator of chromatin opening [15]. Peretinoin (an acyclic retinoid) inhibits HBV replication by inhibiting HDAC1 through the sphingosine metabolic pathway [10]. *In vitro* experiments of this study, PCV2 infection significantly upregulated the protein expression level of Ac-H4 and HAT activity, while decreasing the activity of HDAC. However, after PCV2 infection, the HDAC activity and the protein expression level of Ac-H4 in the 3D4/2 cells were increased and reduced by PNS treatment respectively, but no significant effect was observed on the activity of HAT and the protein expression level of Ac-H3. Therefore, PCV2 infection may upregulate the protein expression level of Ac-H4 by increasing HAT activity and decreasing HDAC activity, and then make the histone acetylation imbalance in 3D4/2 cells. *In vivo*, the protein expression level of Ac-H3 and HAT activity were upregulated in PCV2-infected mice, while HDAC activity was down-regulated. These results suggested that PNS enhanced the scavenging of the free radicals and downregulated the protein expression level of Ac-H3 in the spleens of PCV2-infected mice, which may regulate histone acetylation by inhibiting the activity of HAT. This supposition is supported by the results of a previous study showing that the addition of HAT activators to immune cells infected with bovine herpesvirus 1 (BoHV-1) enhanced viral replication, while the addition of HAT inhibitors inhibited viral replication, indicating that histone acetylation contributes to viral replication [30]. Therefore, the results of our experiments may be attributed to PNS inhibiting the replication of PCV2 by reducing the activity of HAT. Furthermore, the effect of PNS on epigenetic changes may also imply a preventive role in viral infectious diseases. Thus, our findings implicate PNS in the prevention and alleviate of relevant diseases

caused by viruses.

To sum up, PCV2 infection causes changes in antioxidant and epigenetic activities *in vitro* and in mice, and oxidative stress-related factors play an important role in histone modification, indicating a possible relationship between oxidative stress and histone acetylation. PNS regulated the changes in antioxidant activities caused by PCV2 infection and modulated the acetylation of cells by regulating the histone acetylase or deacetylase in immune cells, suggesting that PNS may be used as an effective prevention and alleviate for PCV2 infectious disease.

CONFLICT OF INTEREST. There were no conflicts of interest on any front.

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FIGURE LEGENDS

Fig. 1. The test flow diagrams with *Panax notoginseng* saponins treatment in Porcine circovirus type 2-infected mice.

Fig. 2. The effects of *Panax notoginseng* saponins on the antioxidant activity in 3D4/2 cells with Porcine circovirus type 2 infection.

Notes: A. superoxide anion radicals; B. hydroxyl radicals; C. hydrogen peroxide. Data are presented as mean $\pm$ SD. The difference of shoulder lowercase letters means that the comparison between the two groups is $P < 0.05$, indicating that the two groups have significant differences. The difference of shoulder capitals was $P < 0.01$, indicating that there was a very significant difference between the two groups. The same letter on the shoulder mark was $P > 0.05$, indicating that there was no statistically significant difference between the two groups (The same blow).

Fig. 3. The activities of histone acetylase and histone deacetylase in *Panax notoginseng* saponins treated 3D4/2 cells with Porcine circovirus type 2 infection.

Notes: A. the activity of HAT; B. the activity of HDAC.

Fig. 4. The protein expression levels of histone H3 acetylation or histone H4 acetylation in *Panax notoginseng* saponins treated 3D4/2cells with Porcine circovirus type 2 infection.

Notes: A. western blotting results of Ac-H3, Ac-H4 and β -actin in each group of cells; B. quantitative figure. The protein expression levels of each protein were expressed relative to the protein expression levels of β -actin, and the protein expression levels of the cells in the control group were normalized. The changes in the expression multiples of Ac-H3 and Ac-H4 in the cells in the control group were further observed.

Fig. 5. Effects of *Panax notoginseng* saponins on the antioxidant activity in the spleens of Porcine circovirus type 2 -infected mice.

Notes: A. the scavenging capacity of superoxide anion radicals; B. the scavenging capacity of hydroxyl radicals; C. the content of hydrogen peroxide.

Fig. 6. Effect of *Panax notoginseng* saponins on histone acetylase or histone deacetylase activities in the spleens of Porcine circovirus type 2 -infected mice.

Notes: A. the level of HAT; B. the level of HDAC.

Fig. 7. Effects of *Panax notoginseng* saponins on the protein expression levels of histone H3 acetylation or histone H4 acetylation in the spleens of Porcine circovirus type 2 -infected mice.

Notes: Figure A and B show the western blotting results and quantization of Ac-H3, Ac-H4 and β -actin in the spleens of each group. The expression levels of each protein were expressed relative to the expression levels of β -actin, and the expression levels of the blank control group were normalized to observe the changes in the expression multiples of Ac-H3 and Ac-H4 in the spleens of mice in each group compared with that in the blank control group.