RESEARCH ARTICLE

Pseudorabies Virus Induces Viability Changes and Oxidative Stress in Swine Testis Cell-Line

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ABSTRACT

In this study, we evaluated the association between pseudorabies (PRV) virus-induced viability changes and oxidative stress *in vitro* cultivated swine testis (ST) cells. The kinetic of 2, 12, 24, 36 and 48 h during the cell culture with PRV by using a multiplicity of infection (MOI) of 1 $TCID_{50}$ per cell were adopted. The results suggested a complex relation between cell viability and oxidative stress during PRV infection. In the early stages of PRV infection, the cell viability was higher than the control group, and the state of cellular oxidative stress remained relatively stable. After 24 h, the cell viability began to decrease, and the amount of the cellular malondialdehyde in ST cells increased significantly, and the activities of superoxide dismutase and catalase decreased significantly ($P<0.05$). Meanwhile, the rising concentrations of cellular hydrogen peroxide were detected prior to the changes in cell viability and oxidative stress. In conclusion, the PRV infection of ST cells leads to oxidative stress, and this stress could play a crucial role on the cell viability as the PRV infection time progresses.

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INTRODUCTION

The pseudorabies virus (PRV), usually referred to as Suid Herpes virus 1 or Aujeszky's Disease, is a swine Herpes virus and belongs to the alpha Herpes virus subfamily that causes central nervous disorders in neonatal pigs, respiratory problems in fattening pigs and reproductive failure in breeding pigs, accounting to devastating disease and economic losses throughout the world (Muller *et al*., 2011). PRV has served as a useful model organism in studies of the Herpes virus pathogenesis and molecular biology. Furthermore, it has been commonly used as a neural circuit tracer and as an oncolytic agent. Although the clinical manifestations of PRV have already been described, the pathophysiology of this disease is still not systematically established, especially the molecular mechanisms involved in oxidative stress.

Oxidative stress is a physiological condition of an imbalance between the production and manifestation of reactive oxygen species (ROS) and a biological system's ability to detoxify readily the reactive intermediates or to repair the resulting damage (Xi *et al*., 2012). Under normal physiological conditions, cellular ROS generation is controlled by a large number of antioxidant systems, including superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), vitamins C and E, and other enzymatic and non-enzymatic antioxidants. Excessive ROS accumulation occurs when the pro-oxidant/ antioxidant becomes imbalanced, and it subsequently induces oxidative damage to cellular structures and molecules, such as DNA, proteins and lipid membranes (Valko *et al*., 2007).

Recently, observations of the multiple patho-genetic interactions between ROS and various virus infections have demonstrated that ROS might influence the stages of virus infection (Jackson *et al*., 2011). The association between virus-induced cell proliferation/apoptosis and oxidative stress has been extensively studied for a series of viruses, including Herpes viruses (Gargouri *et al*., 2009; Mathew *et al*., 2010).

PRV is pantropic, because the viron infect many different types of cells, including swine testis (ST) and porcine kidney cells. The ST cell line was previously found as sensitive to the *in-vitro* PRV infection, thus representing an interesting model for the study of

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oxidative stress during PRV infection (Cheung *et al.*, 2000). To gain more insight into the relation between oxidative stress and cell proliferation during PRV infection, we followed the format used by Cheung *et al.* (2000) and evaluated both cell viability and oxidative profiles at 2, 12, 24, 36 and 48 h after viral inoculation. Cell viability was measured by the MTT assay. The oxidative profiles feature the levels of H_2O_2 (one of the most documented ROS produced under oxidative stress conditions) that were found in the activities of two antioxidant enzymes: SOD and CAT. The profiles also include malondialdehyde (MDA) as a marker of lipid peroxidation.

MATERIALS AND METHODS

Reagent: Fetal bovine serum (FBS) and Dulbecco's Modified Eagle Medium (DMEM) were obtained from Gibco (Invitrogen, USA) and the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT), trypsin/EDTA, dimethyle sulfoxide (DMSO), tylosin from Sigma, USA.

Cells and virus source: Stock ($TCID_{50}=10^{-6.9}/0.1$ mL) of standard vaccine strain (PRV-Ra) was provided by Zhaoqing Dahuanong Biology Medicine Co. Ltd (China) and ST cell line were acquired from China Institute of Veterinary Drug Control (China).

Cell proliferation assay: To analyze the ST cell population response to the PRV infection, the authors examined the cell proliferation in the MTT assays. Briefly, ST cells were seeded in the 96-well plate at a density of 10^4 cells (100-µl cell suspension) per well and incubated overnight at $37^{o}C$ in a humidified 5% CO_2 atmosphere. Then, the cells were incubated with PRV-Ra dilution (MOI of 1 $TCID_{50}$ per cell) for 2, 12, 24, 36 and 48 h. At the end of each treatment, 10 µl MTT (5 mg/ml) was added into each well directly, including the control group wells, and then all wells were incubated for 2 h sequentially. After the incubation, the medium was aspirated off and 150 µl DMSO was added to dissolve the formazan crystals. The absorbance of each well was measured at 490 nm in an ELx800 absorbance micro plate reader (Bio-Teke, USA). The data were all expressed as (OD measurement group-OD blank group).

Measurement of oxidative profiles: The ST cells were seeded in the 6-well plate at a density of 3×10^5 cells in a 3-ml cell suspension per well and incubated overnight at $37^{o}C$ in a humidified and 5% CO_2 in the atmosphere. The cells were infected with a PRV–Ra dilution (MOI of 1 $TCID_{50}$ per cell) and incubated for 1 h. The virus suspension was removed and a fresh medium was added to the cells, which was supplemented with 2% FBS. The cells were harvested at a number of PRV-Ra incubation periods: 2, 12, 24, 36 and 48 h after infection. At the end of each treatment, the cells were detached using a trypsin/EDTA solution and centrifuged at 800×g for 5 min. The pellet was re-suspended in 800 µl of deionized water, fixed on the ice, and then lysed by five cycles of ultra-sonication at 300 W with a 1-s pulse time (FS-600 Ultrasonic Processing, Shanghai). The mock-infected and

positive control (100 µMol/L Fe^{2+} treated cells were placed in a MDA assay) were treated in the same way as the infected cells (Gargouri *et al.*, 2009).

The amount of H_2O_2 and MDA as well as the activity of SOD and CAT were determined by using the detection kits obtained from Nanjing Jiancheng Bioengineering Institute China, the assays were prepared by the manufacturer's instructions that have been described previously (Shi *et al.*, 2012). The protein content was expressed as mg per ml, H_2O_2 content was expressed as mMol per mg protein, MDA content was expressed as nMol per mg protein SOD and CAT activity were expressed as Units (U) per mg protein.

Statistical analysis: The data was analyzed with the statistical package SRSS version 11.0. These determinations are reported as mean±SD in the figures and tables. The statistical analyses were done with a Student t-test value to assess the statistical significance of the obtained differences among the groups. A value of P<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

It is well established that virus infections can induce a number of cellular responses in the host and that the majority of these responses help to create a lethal environment for the viruses, controlling their replication and minimizing the deleterious effects of infection. In turn, viruses have developed strategies to induce favorable host cell responses and thus ensure a higher yield of progeny virus for the success of the infection (Mahmood *et al.*, 2012). During their infection stage, many types of virus induce responses that involve alterations in cell properties, in particular the proliferation and apoptosis of the host cell and the presence of oxidative stress. This study showed that ST cells infected with PRV presented significant alterations in their properties. In addition, previous studies have shown that virus-infected cells exhibited a disturbance in cell viability wherein the pathogenic agent seems to be the first responsible and to gain the advantage.

Based on the previous studies, PRV protects infected cells from apoptosis induced by the inner cellular pathway, such as the anti-apoptosis anti-activation caspase-3 pathway and the US3 protein kinase (Aleman *et al.*, 2001; Deruelle *et al.*, 2010). From Table 1, the PRV infection actually promoted cell proliferation during its early stages (12 and 24 h), and the ST cell population became abnormally higher than the mock-infected group at 24 h (P<0.05). Then, the ST cell population began to decrease at 24~36 h of incubation. At 48 h, the viability of the infected cells was significantly lower than the viability of the mock-infected cells (P<0.05). The results reported above all suggested a complex picture of the relation between cell viability and PRV infection.

H_2O_2 is a toxic product of normal aerobic metabolism as well as of pathogenic ROS production that involves CAT and SOD. H_2O_2 plays a crucial role on trigging Kaposi's sarcoma-associated Herpes virus (KSHV) reactivation from latency, and the H_2O_2 scavengers, such as CAT and glutathione, which effectively inhibit KSHV lytic replication in cultures (Ye *et al.*, 2011). H_2O_2 was also found as a key signal for cell proliferation and cell

Table 1: Effect of Pseudorabies virus infection on PRV infection induced alteration in MTT concentration, H_2O_2 content and the activities of superoxide dismutase and catalase production in swine testis cell line

Time		2h	12h	24h	36h	48h
MTT assay	Control	0.313±0.002	0.452±0.035	0.663±0.069	0.894±0.021	1.079±0.124
	PRV	0.31±0.015	0.576±0.029	0.836±0.017*	0.609±0.133*	0.285±0.076*
H_2O_2 content	Control	21.01±0.03	22.75±0.03	22.89±0.24	24.28±0.09	24.75±0.05
	PRV	24.36±0.01	25.22±0.14	28.49±0.06*	34.09±0.04*	35.02±0.04*
SOD Activity	Control	20.34±0.04	19.31±0.05	18.67±0.07	16.73±0.17	17.31±0.42
	PRV	17.07±0.07	18.34±0.11	16.03±0.06	7.24±0.09*	5.11±0.14*
CAT Activity	Control	11.61±0.17	12.74±0.04	9.75±0.03	8.55±0.06	9.32±0.13
	PRV	10.83±0.06	11.36±0.07	10.86±0.04	5.38±0.31*	4.91±0.05*

The effect of PRV infection on ST cells growth properties, H_2O_2 content and the activities of superoxide dismutase and catalase production. 2, 12, 24, 36 and 48 h after PRV incubation, control and PRV infected group were examined. Data are expressed as the mean±SD (n=4). *Shows the difference between the mock-infected and infected group are significant (P<0.05).
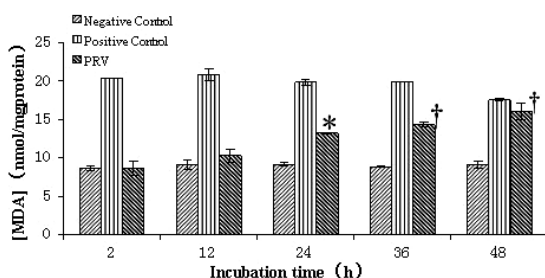

Fig. 1: Pseudorabies virus infection resulted in Malondialdehyde contents of swine testis cell line. *Significant difference (P<0.05); †Highly significant difference (P<0.01).

apoptosis (Iruthayanathan *et al*., 2011). The results showed an increase in the H_2O_2 PRV-infected cells compared with control group, and the difference was accentuated with the extension of PRV infection over the time (Table 1). During the early stages of the PRV infection (2, 12 and 24 h), the difference in H_2O_2 levels between the two groups was not significant (P>0.05), but at 36 and 48 h of incubation, the content of H_2O_2 in the infected cells significantly increased (P<0.05) with a 50% higher concentration than that of the control group. Therefore, H_2O_2 may act as a positive signal that activates PRV infection and induces the ST cell proliferation, but in the final stages of infection, the rapidly increasing level of cellular H_2O_2 could induce the infected cell's apoptosis.

MDA is one of the spontaneous breakdown products of lipid peroxidation, which has been widely employed in biological systems for the assessment of oxidative damage. We detected the amount of MDA with the MDA assay kit used by thiobarbituric acid (TBA) assay. During the period of PRV infection, the cellular MDA content showed rising rates of MDA compared with the control cells (P>0.05), and it became significant after 24 h (P<0.05). In addition, the level of MDA in the infected group was very close to the MDA level of the positive control at 48 h of PRV infection. The results of the MDA showed that oxidative stress occurred in the PRV-infected cells, and the oxidative damage was accentuated with the extension of PRV infection time (Fig.1). This oxidative damage has also been observed during other virus infections. Lassoued *et al*. (2008) demonstrated the oxidative stress during the early stages of an Epstein-Barr (EB) virus infection in B lymphocytes.

SOD and CAT are two important antioxidant enzymes that are omnipresent in every dismutation of cellular H_2O_2, and researchers have proved that this enzyme correlates with virus infection. During the period of hepatitis C virus (HCV) infection, increase in MDA

concentration and impaired SOD and CAT function were observed for both the *in vivo* and *in vitro* models, which indicated that these parameters of cellular oxidative changes were closely related with the findings of Tardif *et al*. (2005). The data of the present study indicated that the SOD and CAT activities changed within the PRV-infected cells in a similar way (Table 1). These difference, however, were statistically significant only after 36 and 48 h of PRV infection (P<0.05), and the activities of SOD and CAT in the ST cells were not declined during the earlier stages of PRV infection (2, 12 and 24 h).

Conclusion: The current investigation demonstrated that *in vitro* PRV infection induced ST cell line proliferation in the early infection period. Moreover, the content of cellular H_2O_2 and MDA increased insignificantly in the early stages, and the activities of these two antioxidants rarely changed. In the later stages of PRV infection, the ST cell viability was decreased, accompanied by significant increase in cellular H_2O_2 and MDA as well as significant decreased in SOD and CAT activities. The results obtained in this study provided better insight supported with data about the interaction between PRV and the ST cell line, which may contribute to elaborate the pathophysiology of PRV infection.

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