In vitro anti-PRV activity of dihydromyricetin from Ampelopsis grossedentata

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ABSTRACT

Pseudorabies (PR) is an acute infectious disease caused by pseudorabies virus (PRV). There are no available drugs due to the emergence of variant of PRV. Dihydromyricetin (DMY) is a flavonoid extracted from Ampelopsis grossedentata (\textit{A. grossedentata}), which has a variety of pharmacological activities. In this study, we aim to investigate the \textit{in vitro} anti-PRV activity of DMY extracted from \textit{A. grossedentata}. MTT assay was used to detect the cytotoxicity and antiviral activity of DMY. The results detected by flow cytometry and qRT-PCR showed that DMY played anti-PRV role mainly by interfering with the process of virus invasion into host cell and inhibiting the occurrence of pyroptosis \textit{in vitro}. This suggested that anti-pyroptosis may be an important antiviral mechanism for DMY which is expected to be a potential anti-PRV drug.

1. Introduction

Pseudorabies virus (PRV) is a swine herpesvirus, which has caused huge economic losses to the pig industry (Delva et al. 2020). Since 2011, the outbreak of pseudorabies (PR) in some farms of China caused by the mutation of PRV strain which has brought great challenges to the prevention and control of PRV. So far, China has taken some measures to prevent and control PRV. However, PRV infection can cause immunosuppression in pigs, resulting in secondary infection of other viruses and bacteria. In addition, due to the emergence of variant of PRV, vaccines don’t have the ability to
completely prevent and control PRV infection (Tang et al. 2017). Therefore, it is of great significance to develop new vaccines and drugs that could inhibit PRV infection.

*Ampelopsis grossedentata* (*A. grossedentata*) is a typical edible plant with important physiological and pharmacological functions. Dihydromyricetin (DMY) is the main active component in *A. grossedentata* (Li et al. 2020), which has antibacterial, anti-inflammatory, antioxidant effects. In addition, DMY has potential application in antiviral field (Feng et al. 2018). However, the effect of DMY on PRV remains unclear.

2. Results and discussions

2.1. The cytotoxicity of DMY to PK15 cell

The cytotoxicity assay was conducted on PK15 cell by MTT method. Cell absorbance value is an index reflecting the number of living cells. There was no difference in the absorbance among DMY treatment groups and control groups (Table S2). It demonstrated that DMY with a concentration of up to 100 μM had no cytotoxicity to PK15 (*p* > 0.05). There was no effect on the cell growth in the vehicle control groups (*p* > 0.05), while could be affected by PRV (*p* < 0.01).

2.2. Anti-PRV activity in vitro

2.2.1. Antiviral activity of DMY before virus addition

DMY has a wide range of biological activities. In recent years, the biological activity of DMY as antiviral has gradually become a research hotspot (Feng et al. 2018). Pre-addition of DMY was thought to inhibit the entry of virus in the early stage of virus infection cycle. PRV attaches to cell membrane through the interaction of glycoprotein C and heparan sulfate proteoglycan, and then binds to specific receptors to stabilize virus and cell interaction (Granzow et al. 1997). The OD570 value of DMY at 100 μM was higher than that in PRV control group and the blocking rate was 17.15% (*p* > 0.05, Table S3). These results indicated that DMY could prevent PRV invasion to a certain extent by inhibiting the binding of PRV to its receptor.

2.2.2. Antiviral activity of DMY after virus addition

Post-addition of DMY is considered to inhibit the entry of virus in the later stage of virus infection cycle. The development of new drugs is of great significance for the prevention and treatment of PRV. It was found that hydroquinone showed high anti-PRV activity by inhibiting the adsorption and internalization of PRV on cells (Fang et al. 2020). Our results showed that the value of DMY at 12.5 μM was higher than that in PRV control group and had an increasing trend in a dose-dependent manner (*p* < 0.05 or *p* < 0.01). Interestingly, the inhibition rate of DMY at 100 μM was higher than 87% (Table S3) indicating that DMY could inhibit PRV.

2.2.3. Entire-infection treatment

The mixed addition of DMY and PRV is thought to result in direct inactivation of virus. The value of DMY at 100 μM was higher than that in PRV control group but had no statistical difference (*p* > 0.05). DMY (100 μM) could inhibit 15.63% PRV replication.
(Table S3) demonstrating that PRV replication could be inhibited directly by DMY. Due to the closed relationships between PRV and human herpes simplex virus type 1 (HSV-1), DMY is expected for the therapy of HSV-1 infections in future.

2.3. Detection of pyroptosis in cell
Previous study has showed that PRV could induce an extensive inflammatory response in mice (Ren et al. 2021). Pyroptosis might be involved in the pathogenesis of PRV which was confirmed by the activation of pyroptosis-related factors NLRP3 inflammasome and IL-1β in vivo and in vitro, respectively (Ye et al. 2021). The result of flow cytometry in this study showed that PI + pyroptotic cells treated by DMY (100 μM) have decreased in a time-dependent manner (p < 0.01) by comparing with the PRV group (Table S4, Figure S1–2). It indicated that DMY posed antiviral activity by reducing pyroptosis in vitro.

2.4. Changes of expression level of genes associated to pyroptosis
The most prominent feature of pyroptosis is that it depends on the activation of caspase-1 and is accompanied by the release of a large number of inflammatory cytokines, i.e., IL-1β and IL-18 (Jorgensen et al. 2017). Activated caspase-1 has a pivot roll in processing and maturing IL-1β/18 (Dipeso et al. 2017). To further detect the influence of DMY on pyroptosis, qRT-PCR was used to detect the mRNA expression levels of several key pyroptosis-related factors. The results showed that the mRNA level of Caspase-1 was decreased (p < 0.05) at 36 hpi DMY treatment in compare to PRV group. GSDMD (Gasdermin D) is a key executor in inflammatory caspase-induced pyroptosis (Jianjin et al. 2018). In our study, the mRNA level of GSDMD was lower than that in the PRV group. It decreased remarkably at 48 hpi (p < 0.01). In addition, the mRNA expression levels of IL-1β and IL-18 were also decreased significantly (p < 0.01) in the DMY groups when compared with PRV group (Table S4 and Figure S3). The results above further proved that DMY could inhibit PRV activity though anti-pyroptosis effect.

3. Conclusion
In conclusion, DMY plays in vitro anti-PRV role mainly by interfering with the process of virus invasion into host cell and decreasing pyroptosis. DMY may be used as an potential compound in future for anti-PRV therapy even might be for the therapy of herpetic infections.

Disclosure statement
No potential conflict of interest was reported by the authors.

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