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SHORT COMMUNICATION

Astragalus Polysaccharide Perseveres Cytomembrane Capacity against Newcastle Disease Virus Infection

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ABSTRACT

Newcastle disease virus (NDV) is highly contagious, leading to the million loss in the poultry industry per year. Unfortunately, there is unknown treatment for ND but the vaccines. The objective of the present study is to evaluate the effect of *Astragalus* polysaccharide (APS) on membrane capacity in response to Newcastle disease virus (NDV) infection and the potential mechanism. Chicken embryo fibroblast cells (CEFs) were exposed to 10^4TCID_{50}/ml NDV, while 150 μg/mL APS were added before/after NDV infection as well as after the mixture. This manner is to mimic preventive, therapeutic, and neutral treatment in clinical practice. Our results shows therapeutic effect of APS was less than preventive effect, while the neutral group was unstable. It shows the significant decrease in silica content, Na^+/K^+-ATPase activity and cell membrane fluidity, and the increase in membrane potential during NDV infection compared with the control groups ($P<0.05$), whereas APS reversed markedly these changes. These suggest APS enhanced the protective effect of CEFs during the early stages of NDV infection. Hence, APS may prevent ND by adding to the feed or nutrition in poultry.

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INTRODUCTION

Newcastle disease virus (NDV) is one member of the genus *Avulavirus* in the family *Paramyxoviridae* that cause highly contagious (Song *et al*., 2015). Unfortunately, there is unknown treatment for ND but the prevention. Thus, the prevention seems critical like vaccination and nutrition.

Astragalus polysaccharide (APS) from the dry roots of *Astragalus membranaceus* has been used since 2000 years ago in traditional Chinese medicine. APS has the antiviral activity and regulation of the immune system (Hafez, 2011). Kallon *et al*. (2013) reported that pretreatment with APS enhanced immunity and inhibited H9N2 in young chicken. However, it is unexplored that whether APS have the effect on membrane capacity during NDV infection. Hurdle *et al*. (2011) demonstrated the breakdown of the immune system has closely associated with the deficiency of cell membrane capacity. Therefore, the action of APS on NDV-induced cell membrane disruption in chicken embryo fibroblasts (CEFs) was investigated in this study.

MATERIALS AND METHODS

Cell lines and virus: CEFs were isolated from ten-day-old chick embryo as the method of Song *et al.*, and cells were incubated in a humidified atmosphere at 37ºC and 5% CO_2. NDV (Mukteswar I strain) was the gift from Prof. Huan-Ran Liu (Harbin Veterinary Research Institute, China). The culture infectious dose ($TCID_{50}$) of virus liquid was calculated by the Reed-Muench method, and $TCID_{50}$ of NDV was $1\times10^{-7.75}$. 100 $TCID_{50}$ diluted with RPMI1640 culture medium was added to CEFs.

Extraction and purification of APS: APS was extracted using Sevag method. Briefly, 100 g dried *Astragalus* were extracted three times with distilled water, and then centrifuged at 1000 g for 10 min and the crude polysaccharide was purified using Sevag method. The sugar of polysaccharide content was 87.5% and dissolved in PBS, filtered through a 0.22 μm Teflon filter and stored at 4ºC. The final concentration of APS for CEF was measured using 3-(4,5-dimethylthiazol-2-yl) (MTT) assay.

Experiment design: CEFs were divided six groups: the control group: RPMI1640 with 10% fetal calf serum (FBS) and 100U penicillin/streptomycin. NDV group (NDV): $100TCID_{50}$/ml was added to CEF plates. APS group (APS): APS was added 150 μg/mL for 3 h at 37ºC and 5% CO_2 prior to NDV addition. Preventive group (preAPS+NDV), 150 μg/mL APS was added for 3 h at 37ºC and 5% CO_2 prior to NDV addition. Therapeutic group (NDV+posAPS): after NDV administration for 3 h, APS

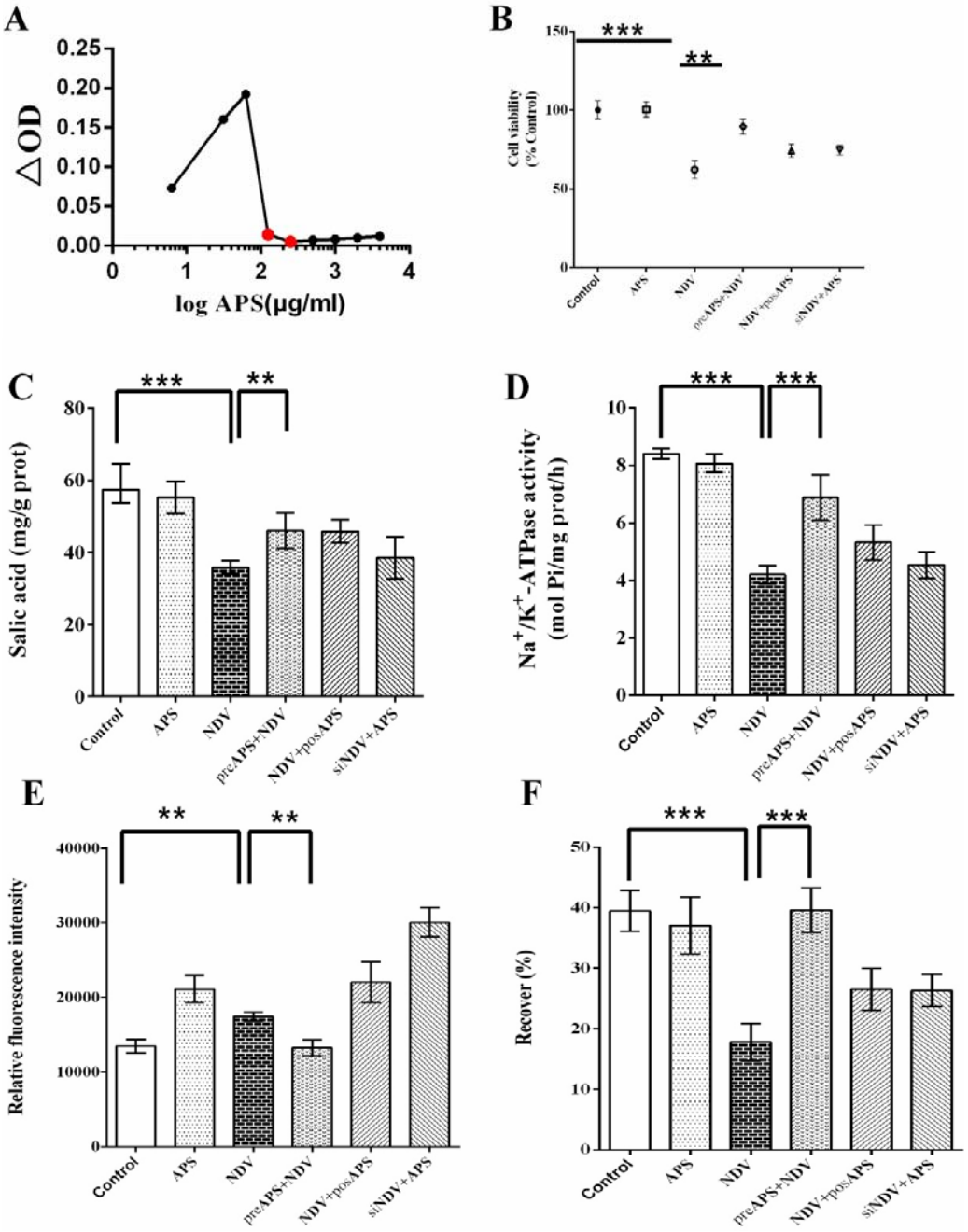

Fig.1: Effects of APS on NDV-induced membrane disruption in CEFs. CEFs were exposed to 10^4TCID_{50}/ml NDV, while 150 μg/mL APS were added before/after NDV infection as well as after the mixture. (A):Cytotoxic effect of APS in CEFs;(B) Cytotoxic activities of APS on the growth in CEFs; (C)Effect of APS on SA content of CEFs; (D) Effect of APS on Na^+/K^+-ATPase activity in CEFs; (E) Effect of APS on membrane potential in CEFs; (F)Effect of APS on membrane fluidity in CEFs. **P<0.05, ***P<0.001. NDV: *Newcastle* disease virus; APS: *Astragalus* polysaccharide; preAPS+NDV: preventive group; NDV+posAPS: therapeutic group; siNDV+APS: neutral group.

was added. Simulaneous addition group (siAPS+NDV): APS were mixed with NDV, incubated for 3 h at 37ºC and then added to CEF. These manners are to mimic preventive effect, therapeutic interventions, and neutralization level in clinical trials.

Parameters studied: Cytotoxic activity was measured by MTT colorimetric assay at 12 h after stimulation as the method of Dyary *et al*. (2014). The total sialic acid (SA) concentration was determined using the commercial kit (Jiancheng Bioengineering Institute, China). The amount of total SA was normalized by total protein content and expressed as milligram per gram of protein.

Na^+/K^+-ATPase kinetic assay was determined as described by Gal-Garber *et al*. (2003). The phosphate was purchased from Sigma-Aldrich. The value of Na^+/K^+-ATPase activity was normalized by total protein content and expressed as mole of Pi per milligram of protein per hour.

For the determination of cell membrane potential, 10 μg/mL fluorescent probe bis-(1,3-dibarbituric acid)-trimethine oxanol (Sigma-Aldrich, USA) was incubated

with CEFs for 10 min at 4ºC. The fluorescence was measured by flow cytometry (BD FACS Aria, USA) and qualitative assessment was performed under BD FACS Aria software. For the determination of cell membrane fluidity, polarity-sensitive fluorescent probe was used to characterize the fluidity of the cell membrane exposure to NDV. In brief, CEFs were incubated with 3 μg/mL 2-6-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino hexanoy-1-hxadecanoyl-sn-glyxero-3-phosphochline (Sigma–Aldrich, USA) for 20 min at room temperature, observed under confocal microscopy, and then recorded the fluorescence intensity before and after bleaching. The fluorescence recovery rate (%) equals to the ratio of fluorescence intensity value after bleaching to initial intensity value before bleaching.

Statistical analysis: Statistical analysis was performed using SPSS 20.0. Data were presented as mean±SEM. The statistical significance of differences among values was determined by one-way ANOVA. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Considering the potential cytotoxicity of APS on CEFs, cytotoxic activity was measured by MTT colorimetric assay at 12 h after stimulation as shown in Fig.1A. Below 100 μg/mL APS improved the growth ratio in CEFs, and thus 150 μg/mL APS was chosen to add when the CEFs was not increased in the present of APS. In addition, the cell viability in the NDV infection group was decreased significantly in comparison with the control group, while 150 μg/mL APS reversed this change in Fig.1B. SA was one of binding sites of NDV (Jain *et al*., 2009). In our study, we have observed that SA content (35.86±1.86) was low during NDV infection compared with control group (57.43±6.29), indicating the loss of SA during virus infection (Jain *et al*., 2009), whereas preAPS+NDV and NDV+posAPS reversed the decrease 28.19% and 27.80%, respectively (Fig.1C). The Na^+/K^+-ATPase, localized in the basolateral-membrane of enterocytes plays a vital role in cell survival and nutrition transport (Gal-Garber *et al*., 2003). The decrease of Na^+/K^+-ATPase activity in the NDV group was presented in Fig.1D; however, the reduce can be ameliorated in preAPS+NDV and NDV+posAPS group, suggesting that cellar energy deficiency without APS and energy supplement with APS when CEFs were infected with NDV. To explore further the action of APS in NDV infection, transmembrane potential was investigated. Treatment with 10^4 $TCID_{50}$/ml NDV increased membrane potential by 29.36% in contrast to the control cells. As shown in Fig.1E, however, pretreatment with APS restored these changes. Interestingly, the transmembrane potential of CEFs was increased both APS and NDV group. By contrast, the increase of membrane potential in APS group was higher than that in NDV group. APS might activate the membrane channel to pass through the membrane readily, and thereby get into cells (Cheng *et al*., 2014a). Despite all these, pretreatment with APS protected effectively from the increase after NDV infection, which APS may switch the recognition site after entry into cells. In addition, the cell integrity was important for the defense of virus invasion. Membrane fluidity was reduced after virus infection, nevertheless, the presence of APS shown a higher recover ratio than that of NDV infection in Fig.1F. These results demonstrated APS could return intact of the membrane fluidity when NDV was invaded and blocked the virus invasion.

Toll-like receptor, such as TLR3, TLR7 and TLR8 may have nearly association with pattern recognition receptors in the innate immunity (Cheng *et al*., 2014b), and thus we cannot rule out whether these receptors or one of them may play a critical role in defense against NDV infection.

Conclusion: Based on these, 150 μg/mL APS maintained SA, Na^+/K^+-ATPase activity and fluidity, restored the cell integrity and thereby prevented the NDV from entering cells during NDV infection. In contrast, therapeutic effect of APS was not as good as preventive effect, and neutral group showed unstable. Therefore, APS may be as a drug or nutrition and used to prevent virus invasion in the early stage of NDV infection, and reduce the loss of poultry.

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Author's contribution: RZ and MG conceived and designed the review. CX, GS and XZ executed the experiment and analyzed cell samples. WZ analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents, and approved the final version.

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