Achyranthes bidentata Polysaccharide Inhibits Salmonella Invasion of Porcine Small Intestinal Epithelial Cells: A Postprint

Authors: Yurong Zhao, Wang Yaodong

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Abstract

This study employed porcine small intestinal epithelial cells (intestinal porcine epithelial cells-1, IPEC-1) as a model to investigate the effects of Achyranthes bidentata polysaccharides (ABPS) on IPEC-1 proliferation, tight junction-related protein mRNA expression, and Salmonella invasion. ABPS at corresponding doses was added to IPEC-1 cell culture medium to achieve final ABPS concentrations of 0, 50, 100, 200, and 400 g/mL in the medium. MTT assay, real-time quantitative PCR, and plate counting method were used to detect the effects of ABPS on IPEC-1. The results showed that: 1) Compared with the control group, ABPS had no significant effect on IEPC-1 proliferation ($P>0.05$). 2) ABPS treatment significantly inhibited Salmonella invasion; with increasing ABPS concentration, its inhibitory effect first increased and then decreased, reaching a peak at a concentration of 50 g/mL, where the number of Salmonella invading the cells was extremely significantly lower than those in the control group and the 200 and 400 g/mL ABPS groups ($P<0.01$). 3) Compared with the control group, both 50 and 200 g/mL ABPS extremely significantly upregulated the relative mRNA expression of IEPC-1 tight junction-related proteins [Ras-related C3 botulinum toxin substrate 1 (RAC1), zonula occludens-1 (ZO-1), Occludin, Claudin-1] ($P<0.01$). It can thus be concluded that appropriate amounts of ABPS can enhance small intestinal mucosal barrier function and inhibit Salmonella invasion by upregulating tight junction-related protein mRNA expression in IEPC-1.

Full Text

Inhibitory Effects of Achyranthes bidentata Polysaccharides on Salmonella Infection in Porcine Intestinal Epithelial Cells

ZHAO Yurong, WANG Yaodong
College of Animal Science and Technology, Hunan Agricultural University; Hunan Co-Innovation Center of Animal Production Safety, Changsha 410128, China

Abstract

This study investigated the effects of Achyranthes bidentata polysaccharides (ABPS) on proliferation, tight junction-related protein mRNA expression, and Salmonella infection in intestinal porcine epithelial cells-1 (IPEC-1). ABPS was added to IPEC-1 culture medium at final concentrations of 0, 50, 100, 200, and 400 g/mL. MTT assay, quantitative real-time PCR, and plate colony counting were used to assess ABPS effects on IPEC-1. The results showed: (1) ABPS had no significant effect on IPEC-1 proliferation compared with the control group ($P>0.05$). (2) ABPS treatment significantly inhibited Salmonella infection, with inhibitory effects increasing then decreasing as ABPS concentration rose. The optimal inhibitory effect occurred at 50 g/mL, where the number of intracellular Salmonella was significantly lower than in the control, 200 g/mL, and 400 g/mL groups ($P<0.01$). (3) Compared with the control, 50 and 200 g/mL ABPS significantly upregulated mRNA expression of tight junction-related proteins including Ras-related C3 botulinum toxin substrate 1 (RAC1), zonula occludens-1 (ZO-1), Occludin, and Claudin-1 ($P<0.01$). These findings indicate that appropriate ABPS concentrations can enhance intestinal mucosal barrier function and inhibit Salmonella infection by upregulating tight junction-related protein mRNA expression in IPEC-1.

Keywords: Achyranthes bidentata polysaccharides; Salmonella; intestinal porcine epithelial cells-1; tight junction

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The intestinal mucosal barrier effectively prevents diffusion of intestinal microbes and toxins to extraintestinal tissues while blocking pathogen invasion, with well-structured tight junctions between epithelial cells being essential for normal barrier and absorptive functions. Tight junctions are protein complexes formed through interactions of transmembrane and peripheral proteins, including Occludin, Claudins, and zonula occludens (ZO) proteins 1-3. Altered expression or localization of these proteins induces cytoskeletal rearrangement and junction disruption, increasing cell permeability and weakening antigen barriers, allowing pathogens to enter the body and trigger intestinal diseases. Salmonella, a Gram-negative bacterium, invades intestinal epithelial cells through induced phagocytosis and survives within Salmonella-containing vacuoles. In swine

production, Salmonella readily infects 2-4 month-old piglets, causing diarrhea and mortality. Previous research demonstrated that Salmonella SL1344 and SARB21 infection of human colon adenocarcinoma cells (Caco-2) reduces Occludin, Claudin-1, and ZO-1 expression, disperses their distribution, increases intercellular spaces, and blurs cell outlines.

Achyranthes bidentata polysaccharides (ABPS) are bioactive polysaccharides extracted from *Achyranthes bidentata* with antioxidant, antitumor, and immunomodulatory properties. Dietary ABPS supplementation reportedly inhibits intestinal pathogen growth and reduces diarrhea incidence in piglets, though its role in regulating tight junction formation remains unclear. Therefore, this study used IPEC-1 cells as a model to investigate ABPS effects on Salmonella infection, providing insights for healthy piglet production and ABPS development.

1.1 Main Materials

IPEC-1 cells and Salmonella 817 strain were kindly provided by Texas A&M University College of Veterinary Medicine. ABPS (purity 99%) was purchased from Wuhan Dongkangyuan Technology Co., Ltd.

1.2 Main Reagents and Preparation

DMEM/F12 medium, 0.25% trypsin, and antibiotic-antimycotic solution were from Hyclone; fetal bovine serum (FBS) and cell cryopreservation medium from Gibco; D-Hank' s buffer and neutral phosphate-buffered saline (PBS) from Biotopped; Trizol (Carlsbad, CA, USA), PrimeScript™ RT reagent Kit with gDNA Eraser (RR047A), and SYBR Premix Ex Taq™ II (RR820A) from TaKaRa (Dalian); epidermal growth factor (EGF) and ITS liquid media supplement (100×) from Sigma.

100 mg/mL ABPS stock solution: 0.500 g ABPS powder dissolved in 5 mL triple-distilled water, sterilized by 0.22 m membrane filtration, aliquoted, and stored at -20°C.

LB liquid medium: 10 g peptone, 5 g yeast extract, and 10 g NaCl dissolved in 950 mL double-distilled water, pH adjusted to 7.0 with 1 mol/L NaOH, volume brought to 1 L, autoclaved at 121°C for 20 min, stored at 4°C.

LB solid medium: Prepared as above with 15 g agar powder added before autoclaving. Plates were poured after cooling to 50-60°C.

Basal medium: 95% DMEM/F12 + 5% FBS + 5 g/L EGF + 1‰ ITS + 100 U/mL penicillin + 100 g/mL streptomycin.

5 mg/mL MTT: 0.5 g MTT dissolved in 100 mL PBS, sterilized by 0.22 m filtration, stored at 4°C protected from light.

1.3.1 Cell Culture

Activated IPEC-1 cells were cultured in basal medium at 37°C with 5% CO . Upon reaching 80-90% confluence, cells were digested with 0.25% trypsin, counted under an inverted microscope, and collected for subculture or experiments.

ABPS Effects on IPEC-1 Proliferation

Cells were seeded at 1×10 cells/well in 96-well plates with 200 L basal medium. After attachment, ABPS was added to achieve final concentrations of 0, 50, 100, 200, and 400 g/mL. Cells were incubated for 12, 24, 48, or 72 h (6 replicates per time point per treatment). MTT solution (20 L) was added and incubated for 4 h. Medium was aspirated, 150 L DMSO added, and plates shaken at low speed for 10 min. Absorbance (OD) was measured at 490 nm using a microplate reader, with OD values representing cell proliferation.

1.3.3 Salmonella Infection of IPEC-1

Salmonella preparation: On the day of cell passage, a single 817 colony was inoculated into 5 mL LB liquid medium and cultured overnight (10-12 h) at 200 rpm, 37°C. Then 10 L was transferred to fresh 5 mL LB medium and cultured (20-24 h) at 37°C. Bacteria were pelleted at 1,000 rpm for 10 min, resuspended in 5 mL basal medium without antibiotics or FBS.

Infection assay: Cells were seeded at 5×10 cells/well in 24-well plates with 1 mL basal medium. After attachment, ABPS was added to final concentrations of 0, 50, 100, 200, and 400 g/mL (5 replicates per treatment) and incubated for 48 h. Medium was aspirated and wells washed twice with PBS. Salmonella suspension (100 L) was added per well, centrifuged at 1,000 rpm for 10 min at room temperature, and incubated for 1 h at 37°C with 5% CO . After aspiration and two PBS washes, gentamicin-containing medium (100 g/mL) without FBS (1 mL) was added and incubated for 2 h. Following three PBS washes, 200 L 1% Triton X-100 lysis buffer was added and incubated for 5 min. Then 800 L PBS was added, cells were pipetted repeatedly, and lysates were transferred to 1.5 mL tubes for 10-fold serial dilution in PBS.

Plate counting: 100 L of diluted lysates (10 ^{3}, 10 , 10 , 3 plates per dilution) were spread on LB plates (Φ9 cm) with a sterile spreader. After absorption, plates were inverted and incubated at 37°C for 18-24 h. Colony-forming units (CFU/plate) were counted.

ABPS Effects on Tight Junction Protein mRNA Expression in IPEC-1

Cells were seeded at 1×10 cells/well in 6-well plates with 2 mL basal medium. After attachment, ABPS was added at final concentrations of 0, 50, and 200 g/mL based on infection results (5 replicates per treatment) and incubated for

48 h. Medium was aspirated and wells washed twice with PBS. Total RNA was extracted using Trizol according to the manufacturer' s protocol.

cDNA synthesis: Performed using TaKaRa PrimeScript™ RT reagent Kit with gDNA Eraser, with products stored at -20°C. Primers were synthesized by Shanghai Sangon Biotech; details are shown in .

Real-time quantitative PCR: SYBR Green I dye method was used with SYBR Premix Ex Taq™ II on a CFX96 system. Reaction mixture (20 L total) contained 10 L SYBR Premix Ex Taq II, 2 L diluted cDNA, 0.8 L each of forward and reverse primers (10 mol/L), and 6.4 L ddH O. Cycling conditions: 95°C for 30 s; 35 cycles of 95°C for 5 s, 60°C for 40 s; melting curve analysis from 60°C to 95°C (0.5°C increments every 5 s). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal reference, and relative gene expression was calculated using the 2^(-ΔΔCt) method.

1.4 Data Processing and Analysis

Data were initially processed using Excel 2007, then analyzed by one-way ANOVA using SPSS 21.0 software, followed by Duncan' s multiple comparison test. Significance was set at $P<0.05$ and highly significant at $P<0.01$.

2.1 Effects of ABPS on IPEC-1 Proliferation

As shown in , IPEC-1 cells treated with various ABPS concentrations for 12, 24, 48, or 72 h showed no significant differences in cell viability among groups at any time point ($P>0.05$).

2.2 Effects of ABPS on Salmonella Infection of IPEC-1

shows that after 48 h ABPS treatment and Salmonella inoculation, CFU counts differed significantly among groups ($P<0.01$). ABPS significantly inhibited Salmonella infection in a dose-dependent manner that first increased then decreased. The 50 g/mL ABPS group showed the strongest inhibition, with significantly fewer intracellular Salmonella than the control, 200 g/mL, and 400 g/mL groups ($P<0.01$), though not significantly different from the 100 g/mL group ($P>0.05$).

2.3 Effects of ABPS on Tight Junction-Related Protein mRNA Expression in IPEC-1

demonstrates that mRNA expression of RAC1, ZO-1, Occludin, and Claudin-1 differed significantly among groups ($P<0.01$). Compared with the control, 50 and 200 g/mL ABPS significantly upregulated expression of all four genes: RAC1 increased by 77% and 48%, ZO-1 by 79% and 64%, Occludin by 125% and 81%, and Claudin-1 by 218% and 140%, respectively. Expression of RAC1,

Occludin, and Claudin-1 was significantly lower in the 200 g/mL group than in the 50 g/mL group ($P<0.01$).

3 Discussion

Numerous studies have demonstrated that ABPS improves intestinal function and immunity. Qin et al. reported that ABPS ameliorates LPS-induced stress effects on intestinal absorption and immune function. Li found that 300-1,200 g/mL ABPS significantly promoted proliferation of porcine intestinal epithelial cells (IPEC-J2) and provided protective effects. However, our results showed no significant effect of ABPS on IPEC-1 proliferation, possibly due to differences in cell lines or culture conditions.

Salmonella is a Gram-negative pathogen causing intestinal infections in animals and foodborne illness in humans, representing one of the most common bacterial food poisoning agents. In pig production, Salmonella readily infects 2-4 month-old piglets with underdeveloped intestinal function and low immunity, causing fever, lethargy, persistent diarrhea, growth retardation, and potentially fatal septicemia. Our findings that ABPS-pretreated IPEC-1 cells resist Salmonella infection align with reports by Chen and Ma et al., who demonstrated that dietary ABPS inhibits intestinal pathogen growth in weaned piglets and that ABPS exhibits antibacterial effects against *E. coli* and *Staphylococcus aureus* in vitro.

Intact tight junctions between intestinal epithelial cells are crucial for normal mucosal barrier and absorptive function. These junctions are protein complexes whose function depends on proper expression and localization of junctional proteins, cytoskeletal proteins, and adherens junction integrity. Altered protein expression or localization damages cell structure, increases permeability, and allows pathogens and toxins to enter circulation, triggering intestinal inflammation. Yu demonstrated that Salmonella SL1344 and SARB21 infection disperses Occludin, Claudin-1, and ZO-1 distribution and reduces their expression in both Caco-2 cell monolayers and rat models, increasing intercellular spaces and blurring cell outlines. RAC1, widely expressed in various tissues, plays a critical role in maintaining, enhancing, and restoring endothelial barrier function. Basal RAC1 activity is essential for forming and maintaining adherens junctions and cytoskeletal structures. RAC1 inhibition disrupts adherens junction complexes and actin cytoskeleton connections. Our results show that ABPS significantly upregulates mRNA expression of tight junction proteins (RAC1, ZO-1, Occludin, Claudin-1) and inhibits Salmonella infection, suggesting ABPS strengthens intestinal mucosal integrity and blocks pathogen invasion by promoting tight junction protein expression.

4 Conclusion

Under our experimental conditions: (1) ABPS at 0-400 g/mL had no significant effect on IPEC-1 proliferation. (2) ABPS-treated IPEC-1 significantly

inhibited Salmonella infection, with inhibitory effects increasing then decreasing as ABPS concentration rose. (3) 50 and 200 g/mL ABPS significantly upregulated mRNA expression of tight junction-related proteins in IPEC-1.

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Note: Figure translations are in progress. See original paper for figures.