Effects of Lentinan on Jejunal Morphological Structure, Epithelial Cell Count, and Tight Junction Protein Expression in E. coli-Challenged Rats: Postprint

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Abstract

This study aimed to investigate the effects of Lentinan on jejunal morphological structure, epithelial cell numbers, and tight junction protein expression in rats challenged with Escherichia coli. Twenty-four SD rats were randomly assigned to four groups (Groups A, B, C, D), with six replicates per group and one rat per replicate. Groups A and B received distilled water, while Groups C and D received distilled water supplemented with 20 g/mL Lentinan. On day 15 of the experiment, Groups B and D were orally administered 1$×10^{10}$ CFU/mL E. coli K88, while Groups A and C received an equal volume of normal saline. On day 18, rats were euthanized by cardiac puncture; jejunum samples were collected for fixation and cryopreservation, paraffin-embedded sections were prepared, and hematoxylin-eosin (HE) staining and periodic acid-Schiff (PAS) staining were performed to measure villus height, crypt depth, villus width, intraepithelial lymphocyte (IEL) count, and epithelial goblet cell (GC) count, and to calculate the villus height-to-crypt depth ratio (V/C). Western blotting was also employed to determine the expression level of the jejunal tight junction protein Occludin. The results showed that there were no significant differences in villus height or villus width among groups ($P>0.05$). Crypt depth in Group C was extremely significantly lower than in Groups A and B ($P<0.01$), and significantly lower than in Group D ($P<0.05$). The V/C ratio in Group C was extremely significantly higher than in Groups A, B, and D ($P<0.01$). IEL count in Group C was extremely significantly lower than in Groups B and D ($P<0.01$), and significantly lower than in Group A ($P<0.05$). Epithelial GC count in Group C was extremely significantly higher than in Groups A and D ($P<0.01$), with no significant difference from Group B ($P>0.05$). Occludin expression level in Group D was markedly higher than in Groups A, B, and

C, while Occludin expression level in Group B was higher than in Group A. In conclusion, supplementation of Lentinan in rat drinking water can improve jejunal morphological structure, enhance resistance to E. coli infection, and promote Occludin expression.

Full Text

Effects of Lentinan on Morphology Structure, Epithelial Cell Number and Tight Junction Protein Expression in Jejunum of Rats against E. coli

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Abstract

This study investigated the effects of lentinan on jejunal morphology structure, epithelial cell number, and tight junction protein expression in rats challenged with *Escherichia coli* (E. coli). Twenty-four Sprague-Dawley (SD) rats were randomly divided into four groups (A, B, C, and D) with six replicates per group and one rat per replicate. Rats in groups A and B received distilled water, while rats in groups C and D received distilled water supplemented with 20 μg/mL lentinan. On day 15 of the experiment, rats in groups B and D were orally administered 1$×10^{10}$ CFU/mL E. coli K88, while rats in groups A and C received the same volume of normal saline. On day 18, heart blood samples were collected, and jejunal tissues were harvested for fixation and freezing. Paraffin sections were prepared for hematoxylin-eosin (HE) staining and periodic acid-Schiff (PAS) staining. Villus height, crypt depth, villus width, number of intraepithelial lymphocytes (IEL), and epithelial goblet cells (GC) were measured, and the ratio of villus height to crypt depth (V/C) was calculated. The expression of tight junction protein Occludin in the jejunum was determined using Western blot.

The results showed no significant differences in villus height or villus width among all groups ($P>0.05$). The crypt depth in group C was significantly lower than that in groups A and B ($P<0.01$) and significantly lower than that in group D ($P<0.05$). The V/C ratio of group C was significantly higher than that of groups A, B, and D ($P<0.01$). The IEL number in group C was significantly lower than that in groups B and D ($P<0.01$) and significantly lower than that in group A ($P<0.05$). The epithelial GC number in group C was significantly higher than that in groups A and D ($P<0.01$), with no significant difference compared to group B ($P>0.05$). The Occludin expression in group D was markedly higher than that in groups A, B, and C, and the Occludin expression in group B was higher than that in group A. In conclusion, supplementing drinking water

with lentinan can improve jejunal morphology, enhance resistance to E. coli, and promote Occludin expression in rats.

Keywords: lentinan; *Escherichia coli*; jejunum; tight junction protein; rats

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1. Materials and Methods

1.1 Experimental Design Twenty-four healthy SD rats with an average body weight of 50.52 g were randomly allocated into four groups (A, B, C, and D) with six replicates per group and one rat per replicate. Groups A and B received distilled water, while groups C and D received distilled water supplemented with 20 μg/mL lentinan at a concentration of 0.5 mg/mL. On day 15 of the experiment, groups B and D were orally administered 2 mL of E. coli K88 suspension at 1$×10^{10}$ CFU/mL, while groups A and C received an equivalent volume of normal saline. All rats were housed in individual ventilated cages (IVC) under controlled conditions (temperature $25\$\pm2°C, relativehumidity50\pm\$5\%$) with ad libitum access to feed and water.

1.2 Sample Collection and Processing On day 18, rats were euthanized and jejunal segments approximately 3–4 cm in length were collected. Tissues were fixed in 4% paraformaldehyde for 24 hours, dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin. Sections (4 μm thick) were prepared for HE and PAS staining. Some tissues were snap-frozen in liquid nitrogen and stored at -80°C for Western blot analysis.

1.2.1 Histological Staining Paraffin-embedded sections were deparaffinized, rehydrated, and stained with HE and PAS according to standard protocols for morphological observation.

1.2.2 Morphometric Analysis Villus height, crypt depth, and villus width were measured using Motic 2.0 image analysis software. Images were captured with a microscope and analyzed using Image-Pro Plus 6.0 software. The V/C ratio was calculated for each sample. Six randomly selected fields were examined per section.

1.2.3 Intraepithelial Lymphocyte (IEL) Counting IELs were counted in three PAS-stained sections per sample at 10$×$20 magnification. Six fields were randomly selected per section, and IEL numbers were expressed as percentages.

1.2.4 Goblet Cell (GC) Counting GCs were counted in three HE-stained sections per sample at 10$×$20 magnification. Six fields were randomly selected per section, and GC numbers were expressed as percentages.

1.2.5 Western Blot Analysis Total protein was extracted from jejunal tissues and quantified using the BCA method (Thermo). Protein samples (30 μg) were separated by SDS-PAGE and transferred to PVDF membranes. After blocking, membranes were incubated with primary antibodies against Occludin, followed by HRP-conjugated secondary antibodies. Protein bands were visualized using an AlphaImager 2200 system (Alpha Innotech) and quantified by densitometry.

1.3 Statistical Analysis Data were analyzed using SAS 9.4 software. The PROC MIXED procedure was used for analysis of variance, with results expressed as means ± SEM. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

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2. Results

2.1 Effects of Lentinan on Jejunal Morphology Structure As shown in and [Figure 1: see original paper], no significant differences were observed in villus height or villus width among all groups ($P>0.05$). The crypt depth in group C was significantly lower than that in groups A and B ($P<0.01$) and significantly lower than that in group D ($P<0.05$). The V/C ratio in group C was significantly higher than that in groups A, B, and D ($P<0.01$). There was no significant difference in V/C ratio between groups A and B ($P=0.64$) or between groups A and D ($P=0.07$). The V/C ratio in group C was significantly higher than that in groups A and B ($P<0.01$) and significantly higher than that in group D ($P<0.05$).

2.2 Effects of Lentinan on Epithelial Cell Numbers As shown in , [Figure 2: see original paper], and [Figure 3: see original paper], the IEL number in group C was significantly lower than that in groups B and D ($P<0.01$) and significantly lower than that in group A ($P<0.05$). The GC number in group C was significantly higher than that in groups A and D ($P<0.01$), with no significant difference compared to group B ($P>0.05$). The GC number in group C was significantly higher than that in groups A and D ($P<0.01$), while the difference between groups C and B was not significant ($P>0.05$).

2.3 Effects of Lentinan on Tight Junction Protein Expression Western blot analysis revealed that Occludin expression in group D was significantly higher than that in groups A, B, and C ($P<0.01$), while Occludin expression in group B was higher than that in group A ($P<0.05$). No significant difference was observed between groups A and C ($P>0.05$).

Effects of lentinan on jejunal morphology structure and epithelial cell number of rats against E. coli

Items	Villus height (μm)	Crypt depth (μm)	Villus width (μm)	V/C	Intraepithelial lymphocyte (%)	Goblet cell (%)
Groups						
P-value						
Lentinan	0.56	<0.01	0.13	<0.01	<0.01	<0.01
E. coli	0.64	<0.01	0.07	<0.01	<0.01	<0.01
Lentinan×E. coli	0.18	0.56	0.13	0.13	0.13	0.13

Items	Villus height (μm)	Crypt depth (μm)	Villus width (μm)	V/C	Intraepithelial lymphocyte (%)	Goblet cell (%)
A	529.86$±58.59|234.07±20.69 ^{Bb}|138.74±21.85|2.27±0.10 ^{BCb}|21.67±4.66 ^{ABa}|15.70±1.47 ^{Cc}||B|537.29±58.59|293.56±42.00 ^{Aa}|133.98±22.28|1.85±0.20 ^{Cc}|25.81±2.70 ^{Aa}|23.84±1.35 ^{Bb}||C|586.04±161.64|176.30±40.68 ^{Cc}|117.54±16.85|3.33±0.50 ^{Aa}|15.11±4.33 ^{Bb}|25.47±3.02 ^{Bb}||D|545.57±99.68|222.05±35.71 ^{BCb}					

Note: In the same row, values with different small letter superscripts indicate significant difference (P<0.05), and different capital letter superscripts indicate highly significant difference (P<0.01), while the same or no letter superscripts indicate no significant difference (P>0.05). The same notation applies below.

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3. Discussion

The intestinal mucosal barrier serves as the first line of defense against pathogenic bacteria and toxins. Lentinan, a 1,3-β-glucan polysaccharide extracted from *Lentinus edodes*, has been reported to possess immunomodulatory and anti-inflammatory properties [1-2]. Previous studies have demonstrated that lentinan can enhance intestinal immune function and maintain mucosal integrity [3-4]. The present study investigated the protective effects of lentinan on jejunal morphology and barrier function in E. coli-challenged rats.

Our results showed that lentinan supplementation significantly reduced crypt depth and increased the V/C ratio, indicating improved intestinal morphology and enhanced absorptive capacity. The V/C ratio is a critical indicator of intestinal health, with higher values suggesting better nutrient absorption and mucosal integrity [5-6]. The reduction in crypt depth may reflect decreased cell turnover due to reduced inflammation and epithelial damage [7].

The intestinal epithelium contains various cell types, including IELs and GCs, which play crucial roles in immune surveillance and mucosal protection. IELs are important components of the gut-associated lymphoid tissue, while GCs secrete mucin to form a protective barrier [8-9]. In this study, lentinan treatment decreased IEL numbers while increasing GC numbers, suggesting a shift toward enhanced mucosal defense rather than inflammatory responses. This is consistent with previous reports that lentinan can modulate immune cell distribution and function [10-11].

Occludin is a key tight junction protein that regulates paracellular permeability and maintains epithelial barrier integrity [12-13]. Our Western blot results demonstrated that E. coli challenge alone (group B) increased Occludin expression compared to the control (group A), possibly as a compensatory response to infection. However, the combination of lentinan and E. coli (group D) showed the highest Occludin expression, indicating that lentinan can further enhance tight junction protein expression under pathogenic challenge. This suggests that lentinan strengthens the intestinal barrier by upregulating Occludin, thereby reducing pathogen translocation [14-15].

Previous studies have shown that polysaccharides can protect intestinal epithelial cells from oxidative stress and inflammation [16]. The mechanism may involve activation of signaling pathways that promote cell proliferation and differentiation while suppressing inflammatory cytokine production [17]. The enhanced Occludin expression observed in our study supports the notion that

lentinan reinforces tight junction integrity, which is essential for preventing bacterial invasion and maintaining gut homeostasis [18].

In conclusion, dietary supplementation with lentinan improves jejunal morphology, modulates epithelial cell populations, and enhances tight junction protein expression in rats challenged with E. coli. These findings suggest that lentinan could be a promising feed additive for improving intestinal health and disease resistance in animals.

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