Exploring the Therapeutic Potential of Paenibacillus-Derived Exopolysaccharides in Colorectal Cancer: A Cellular and Inflammatory Perspective

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Abstract: This study seeks to explore the possibility of discovering a novel therapeutic agent for colorectal cancer. The exopolysaccharide (EPS-J12) from Paenibacillus spp. PYQ-J12 was used as the experimental subject. Caco-2 cells were used to simulate impaired intestinal epithelial cells, and TNF-α was used to cause inflammation of the cells, which was expected to investigate the reparative functions of EPS on inflamed, damaged Caco-2 cells and its influence on the expression levels of different inflammatory factors. Cell viability was used as an indicator to evaluate the reparative effects of the polysaccharide. The expression levels of different cytokines, including Interleukin 6 (IL-6), IL-8, IL-10, IL-1β, and Interleukin 12A (IL-12A), were tested. The findings indicated that EPS has a certain repair effect on inflammatory damage and can cause a reduction in the expression levels of inflammatory factors, thereby controlling inflammation. Therefore, it has the potential to become a new therapeutic option.

1. Introduction

Human colorectal cancer (CRC) has become an increasingly common disease worldwide and it also contributes to global mortality. Although the pathogeny of CRC remains unclear, it can be affected by the gut microbiota, intestinal inflammation, obesity, smoking, diabetes, some living habits, etc. Also, some pathological changes of diseases like familial adenomatous polyposis (FAP), ulcerative colitis (UC), and inflammatory bowel disease (IBD), have been confirmed to be related to colorectal cancer. Yet now the therapy of CRC mainly concludes surgery, adjuvant chemotherapy, and chemoradiation. Currently, the side effects of chemotherapy drugs are quite severe, thus we need to find a safer and more proper anti-cancer drug.

Over the past few decades, attention has surged regarding the role of microbial polysaccharides in various disease processes, including cancer. Microbial polysaccharides are complex carbohydrates produced by microorganisms, such as bacteria and fungi. These polysaccharides show multiple biological functions, containing antioxidative, anti-inflammatory, and antitumor properties. These polysaccharides can affect intestinal cells in multiple ways, such as influencing the release of inflammatory cytokines and modulating mRNA expression levels via different signaling pathways. Furthermore, several studies have proven that microbial polysaccharides may be crucial to intestinal health. It has been proposed that these polysaccharides may affect the composition of the intestinal microbiota, boost the immune function, and manage the inflammatory environment in the colon.

Paenibacillus spp. are a group of Gram-positive, spore-forming bacteria that have been well-researched for their exopolysaccharide (EPS) production capabilities. These EPSs are high-molecular-weight polysaccharides manufactured by microorganisms and serve as a key factor in biofilm formation, stress tolerance, and protection against environmental challenges. Recently, scientists have recognised EPSs potential in repairing diverse damage types, such as wound healing and skin repairing. EPS from Paenibacillus spp. are rich in sources, easy to obtain, and have potential repairing effects, which may become one of the drugs for repairing damaged cells in CRC.

This study focuses on examining the reparative properties of EPS derived from Paenibacillus spp. PYQJ12 (EPS-J12) on Caco-2 cells stimulated by TNF-α damage as well as its potential in improving TNF-α-induced Caco-2 cell inflammatory response. The cytokine TNF induces inflammatory mediators, including cytokines and lipid mediators. Furthermore, by triggering chemokines and adhesion molecules, the activation of endothelial cells attracts inflammatory cells, rendering it suitable for application in the modeling of inflammation. We hypothesize that EPS-J12 treatment can repair Caco-2 cell induced by TNF-α damage and attenuate the inflammatory response, thus preserving intestinal barrier function to achieve therapeutic effect.

In this study, we established a Caco-2 cell damage model by exposing cells to TNF-α. Subsequently, we evaluated the repairing influence of EPS-J12 on TNF-α-induced cell damage by assessing cell viability.
Additionally, we examined the changes in inflammatory markers, such as cytokine production, to determine the impact of EPS-J12 on inflammatory Caco-2 cell.

2. Methods

2.1. Reagents and materials

Fluorescent markers were sourced from Beyotime Institute of Biotechnology, Shanghai, PRC. Sigma-Aldrich, St. Louis, MO, United State provided monosaccharide standards and MTT. All other materials met analytical standards.

The polysaccharides used in this study were derived from the strain PYQ-J12. *P. polymyxa* PYQ-J12, characterized by its slimy colonies, was originated from wood powder gathered in Zhejiang Province, China.

2.2. Extraction of the EPS

From the beginning, the PYQ-J12 bacteria were grown on solid medium and maintained at 30°C for incubation throughout the night in a bacterial incubator. The following day they were removed from the solid medium and placed in fermentation liquid medium (saccharose 50 g/L, tryptone 5 g/L, yeast flour 1 g/L, Na₂HPO₄·12H₂O 3 g/L). After 24 hours of fermentation, the resulting fermentation broth was centrifuged for 15 mins at 4°C and 4000 × g in a refrigerated centrifuge to remove the bacteria. Three times the volume of anhydrous ethanol was blended with the supernatant and precipitated at 4°C till the second day, then the crude EPS was obtained. Further purification and extraction processes consist of two steps. EPS purification was conducted on a DEAE-52 anion exchanger (26 mm x 300 mm) using purified water without ions, 0.1 M along with 0.3 M NaCl for elution at a stream speed of one ml/min. Eluted samples were gathered and analyzed through the phenol-sulfuric acid technique at a wavelength of 490 nm. The isolated fractions were refined on a Sephadex G-100 column (16 mm × 600 mm), using demineralized water for elution. Fractions were collected, dialysed and lyophilised, and the polysaccharides were collected.

2.3. Determine the solution concentration of TNF-α and EPS-J12 required for the experiment.

2.3.1 Cell resuscitation, passaging and cell seeding

The Caco-2 cells frozen in liquid nitrogen were taken out and resuscitated, and then a mixed medium of fetal bovine serum and the cells were treated with DMEM high glucose medium. Subsequently, the cells underwent cultivation in a cell culture incubator for one day.

While the cells were subcultured, 20 μL of the trypsinized cell suspension was taken for cell counting, and the cell suspension was diluted according to the counting results. The cell suspension needs to be diluted to ensure that there are at least 5000 cells in each well of the 96-well microplate, and 100 μL of the suspension is pipetted to every well. The 96-well microplate was then grown in a cell culture incubator for at least 24 hours at 37°C to ensure cell adhesion and growth.

2.3.2 Dosing treatment

The medium was used to adjust the concentration of TNF-α to 10, 20, 50, 100, 200, 500, 1000, 2000 and 5000ng/L. The total carbohydrate levels in EPS were assessed via the phenol-sulphuric acid technique, wherein D-glucose was used as the reference. The polysaccharide concentration was adjusted to 100, 200, 500, 1000 and 2000 μg/mL by mixing with the medium. The 96-well late was taken and the old medium was removed. Following the washing of each well in the plate through PBS, 100 μL medium with varying concentrations of TNF-α or polysaccharides was dispensed into every well, and then the 96-well plate was further placed in a cell culture incubator for cultivation. Each group was set up with three parallel groups.

2.3.3 Viability was assayed by MTT assay

After the 96-well plate was taken out, the dosing medium in the well plate was discarded, then the well plate was washed with PBS buffer, and then 100 μL PBS was pipetted to each well. Then the MTT mother liquor and DMEM high sugar medium were mixed at a ratio of 1:9 by volume. Subsequently, the well plate was inoculated with 100 μL of diluted MTT solution in each well, and then the plate was put into the cell culture chamber. Following 4 hours of cell cultivation, the orifice plate was removed from the incubator. The MTT solvent was discarded, and subsequently, 150 μL of DMSO was introduced into every well. The 96-well plate after adding DMSO solution was vibrated and mixed for 10 minutes and put into a microplate reader, the optical density of the well plate was assessed at 570 nm, and the data was recorded.

Based on the experimental results, 1000 ng / mL was finally selected as the concentration of TNF-α used in subsequent experiments to exclude the effect of TNF-α.

2.4. Investigating the repair effect of EPS-J12 on cell damage and the improvement of inflammatory response

2.4.1 Cell viability assay of drug-treated cells

Cells received treatment with a TNF-α solution at 1000 ng/mL and EPS solutions at doses of 0, 100 and 500 μg / mL in the mentioned way, after which cell viability was evaluated through the MTT test.

2.4.2 Determination of inflammatory factors

RNA was extracted following the provided guidelines with the storage temperature of -80 °C after extraction. The levels and quality of RNA specimens were assessed using the Nanodrop ultra-microphotometer. RNA
underwent reverse transcription according to kit instructions and the obtained cDNA was stored at -20 °C. Primers, templates, and other reagents were added to the PCR tube based on the kit instructions. The factors that needed to be measured were IL-10, IL-1β, IL-6, IL-8, IL-12A. PCR procedure was as follows: hold stage: 94 °C, 30s; PCR stage: 94 °C, 5s; 60 °C, 15s; 72 °C, 10s; melting curve phase: 95 °C, 15s; 60 °C, 1min; 95 °C for 15s. The expression fold of inflammatory factors was calculated by the ΔΔCT method.

2.5. Statistical analysis
Group differences were analysed using ANOVA and Duncan’s test through SPSS Statistics 23 (IBM Corp., Armonk, New York, USA), with p<0.05 considered statistically relevant.

3. Outcomes and commentary
The focus of this research was to look into the protective effect of EPS-J12 on Caco-2 cells with inflammatory injury, with the cell survival rate and the expression level of inflammatory factors as the main reference index. At present, relevant studies have shown that other strains, such as the exopolysaccharide, in the genus Bacillus species have high antioxidant activity and anti-cancer effects. However, relatively few research has been done into the impacts of EPS-J12 generated by PYQ-J12 strain on intestinal cells, and this experiment provides a possibility to increase drugs for the repair of intestinal cells.

In this study, Caco-2 cells were used to simulate a monolayer of intestinal cells, and the inflammation-associated cytokine—tumor necrosis factor-α (TNF-α) was used as an inflammatory factor to establish a model of intestinal epithelial cells impaired by inflammation.

3.1. TNF-α and EPS concentration required for the experiment
During the initial stages of the study, appropriate concentrations of TNF-α and polysaccharides should be determined to prevent improper concentrations from affecting cell viability, which may subsequently impact the experimental results.

The figure demonstrates a significant statistical difference in cell viability between TNF-α concentrations of 500 ng/L and 1000 ng/L, which means TNF-α at 1000 ng/L concentration would have a damaging effect on cells (Fig 1 B). These concentrations of EPS-J12 did not significantly affect cell viability (Fig 1 A). Based on the results, we selected TNF-α at a level of 1000 ng/mL as the experimental solution concentration, while the EPS concentrations used in the experiment were 100 μg/mL and 500 μg/mL.

3.2. Repairing effect of EPS-J12 on Caco-2 cell damage caused by TNF-alpha
According to the figure (Fig 2), cell viability in module-building cells is lower relative to the untreated control group, indicating the efficacy of the inflammatory model. Relative to the inflammatory model cells, the cell viability of the group with EPS-J12 increased and was statistically significant. The cell viability values for the groups treated with 100 and 500 μg/mL EPS were 14.46% and 14.58% higher, respectively, compared to the inflammatory group. Therefore, it can be preliminarily inferred that EPS-J12 has a certain repair effect on Caco-2 cell damage caused by TNF-α.
3.3. Repairing impact of EPS-J12 on the inflammatory response of Caco-2 cells induced by TNF-α

3.3.1 The expression level of IL-6
As pictured (Fig 3 A), IL-6 expression in cells of the inflammation model was much greater than that of the control set. Relative to the model of inflammation, IL-6 expression was reduced in the EPS-12 supplemented group, by 18.56% and 43.40% in the groups with the addition of 100 μg/mL as well as 500 μg/mL, respectively.

3.3.2 The expression level for IL-8
Competed with the IL-8 level in the blank control group, the IL-8 level was higher in the inflammatory model cells (Fig 3 B). After the addition of EPS-J12, the expression of IL-8 was substantially reduced. The group treated with 100 μg/mL demonstrated a decrease in 34.22%, while the group received the treatment of 500 μg/mL experienced a reduction of 36.85%.

3.3.3 The expression level for IL-10
As can be seen (Fig 3 C), the expression level for IL-10 in the inflammatory model was much more reduced than that in the blank group, but there was no large significance. After the addition of EPS-J12, its expression level increased significantly, 636.21% (group with 100 μg/mL EPS) and 783.46% (group with 500 μg/mL EPS) respectively.

3.3.4 The expression level of IL-1β
The figure shows that IL-1β is significantly elevated in the inflammatory model group (Fig 3 D); After adding EPS-J12, IL-1β expression levels in the polysaccharide group decreased by 13.83% in 100 μg/mL group and 49.96% in the group 500 μg/mL group.

3.3.5 The expression level of IL-12A
As shown in the figure (Fig 3 E), the level of IL-12A expression was much elevated in the TNF-alpha-induced-inflammatory model versus the blank control, and the level of interleukin 12A expression in the EPS-added cells decreased significantly. After treatment, the IL-12A expression level decreased by 30.90% in 100 μg/mL group and by 49.96% in the group 500 μg/mL.

These results are the expression level of five inflammation-related markers (Fig 3), including proinflammatory factors as well as anti-inflammatory factors. Among them, proinflammatory factors like interleukin 6 and interleukin 8 were notably raised in inflammatory group, indicating inflammation development. IL-6 is pivotal in the acquired immunity, enhancing antibody generation and the growth of effector t-cell, Additionally, synthesising acute-phase proteins, which comprises C-reactive protein and serum amyloid A, is induced by IL-6. Therefore, the level of its expression reflects the severity of inflammation to some extent. IL-8 possesses the ability to attract and activate neutrophils, which makes it an inflammatory mediator. In addition, in TNF-stimulated cells, the levels of expression for IL-8 and its associated factors will increase. However, in the group with a mixture of TNF-alpha and polysaccharide, IL-6, IL-8 and others’ levels decreased, suggesting that EPS-J12 inhibited some proinflammatory factors. Besides, the concentration of the anti-inflammatory cytokine IL-10 is greatly reduced in inflammation model. Its expression level increases after the polysaccharide, but lacks statistical significance, there may be a protective effect of anti-inflammatory factor, which requires further experiments.

Fig 3 Expression levels of five cytokines. A. IL-6 level. B. IL-8 level. C. IL-10 level. D. IL-1β level. E. IL-12A level. Significant deviations from the controls are denoted by # p < 0.05, ## p < 0.02, ### p < 0.01; * p < 0.05, ** p < 0.02.

The EPS manufactured by probiotic Streptococcus thermophilus has previously been shown to be capable of attenuating inflammation in colonic tissues and down-regulating the production of pro-inflammatory indicators, including TNF and IL-6, in a DSS-induced mouse model for ulcerative colitis, but cellular experiments to validate this have been lacking. This study, on the other hand, furnishes more comprehensive experimental evidence on the potential anti-inflammatory effect of EPS, albeit from a distinct source.

Chronic inflammatory conditions lead to oxidative stress-related DNA damage, potentially activating genes that promote tumors and deactivating those that suppress them. The extent of inflammation is associated with the amount of dysplasia. And dysplasia is one of the common precancerous lesions of colon cancer. In colon cancer, inflammation manifests in three distinct forms: chronic inflammation that occurs before tumor development, inflammation triggered by the tumor itself,
and inflammation induced by treatment. If polysaccharides are effective in mitigating the inflammatory response, then they are not only expected to play a therapeutic role, but may also show potential benefits in improving prognosis and preventing disease; reducing cancer risk by decreasing the damage caused by oxidative burden.

Previous researches have demonstrated that polysaccharides are capable of triggering apoptosis in cancer cells by modulating cell signalling pathways, or act as anticancer agents by blocking the cell cycle of tumour cells. However, these potential drug sources may be difficult to obtain, and are not as readily available and low-cost as Paenibacillus spp, which has the potential to be used as a drug on a wide scale. The preliminary evidence of the inflammation-reducing benefits in improving prognosis and preventing disease; reducing cancer risk by decreasing the damage caused by oxidative burden.

The preliminary evidence of the inflammation-reducing effects of EPS-J12 has been established. However, further research is required to determine whether it exerts an effect on intestine epithelial cells damaged by inflammation through the Caco-2 cell inflammation model. The findings suggest that EPS-J12 may improve the survival rate of cells by reducing inflammation. Moreover, EPS-J12 was found to suppress the generation of mediators that promote inflammation like interleukin 6, IL-8, IL-12A, and interleukin 1β, simultaneously enhancing the generation of the anti-inflammatory factor IL-10. However, the specific mechanistic process still needs further exploration.

A limitation of this study is its somewhat superficial exploration of these processes. While we have established the reparative impact of EPS-J12 on cells damaged by inflammation, the specific molecular pathways involved remain to be elucidated. Additionally, future research should extend to examining the effects of EPS-J12 on cancer cells, to determine whether its impact differs from that on inflammation-damaged cells. Despite these limitations, this study opens new avenues for research and potentially novel therapeutic strategies.

4. Conclusion

In this experiment, EPS-J12 showed a potential protective effect on intestinal epithelial cells damaged by inflammation through the Caco-2 cell inflammation model. The findings suggest that EPS-J12 may improve the survival rate of cells by reducing inflammation. Moreover, EPS-J12 was found to suppress the generation of mediators that promote inflammation like interleukin 6, IL-8, IL-12A, and interleukin 1β, simultaneously enhancing the generation of the anti-inflammatory factor IL-10. However, the specific mechanistic process still needs further exploration.

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Reference


