JOURNAL OF EUROPEAN ACADEMY OPEN UNIVERSITY OPEN UNIVERSITY

Vol. 2, No. 6, 2026 1
Research Article

Cytological Evaluation of Sea Cucumber Fucoidan Inhibiting the Activity of Helicobacter pylori in vitro

Yongjun Sun[1], Wenming Ju[1*], Huali Wang[2], Yuemeng Wang[3], Xiaowei Nie[4], Lingling Yu[5], Lingli Zhang[4], Xiaoyan Li[2], Qi Huang[3]

[1] Homey Group Co., Ltd.

[2] Shandong Drug and Food Vocational College

[3] Yantai Institute of Technology

[4] Weihai Ocean Vocational College

[5] Weihai Vocational College

[*]Correspondence: Wenming Ju, Homey Group Co., Shazui Zi, Ltd, Hushan Town, Rongcheng City, Shandong Province, China, 264305.

Project Number: 2023CXPT087；2025TSGCCZZB0717；YSTY20250001；

Abstract: Objective: To study the inhibitory effect of sea cucumber fucoidan on Helicobacter pylori (H. pylori) in vitro and its cytological mechanism, laying a foundation for the further development of natural anti-H. pylori drugs. **Methods:** Sea cucumber fucoidan was prepared by water extraction and alcohol precipitation. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against H. pylori standard strains and clinical drug-resistant strains were determined using the micro-dilution method. Human gastric mucosal epithelial cells (GES-1) were used as experimental subjects and divided into different concentration groups of sea cucumber fucoidan, an H. pylori infection group, and a negative control group. Cell viability was measured using the CCK-8 method; Giemsa staining was used to observe changes in cell morphology; and the expression levels of inflammatory cytokines IL-1β and TNF-α were detected by ELISA. **Results:** Sea cucumber fucoidan effectively inhibited the growth of H. pylori. The MIC for the standard strain and clinical drug-resistant strain was 18 mg/mL and 36 mg/mL, respectively, while the MBC was 36 mg/mL and 72 mg/mL, respectively. Compared with the H. pylori infection group, at the same time point, the sea cucumber fucoidan treatment group showed higher cell survival rates, lighter degrees of cell damage, and lower levels of IL-1β and TNF-α ($P < 0.05$), which gradually decreased as the concentration of sea cucumber fucoidan increased. **Conclusion:** Sea cucumber fucoidan can inhibit the reproduction of H. pylori in vitro, reduce the possibility of gastric mucosal epithelial cell damage and inflammatory reactions caused by H. pylori, and has great potential to be developed into a novel anti-H. pylori drug.

Keywords: Sea cucumber fucoidan; Helicobacter pylori; In vitro inhibition; Cytological evaluation

Introduction

Helicobacter pylori (Hp) is a class of spiral-shaped, microaerophilic bacteria with strict requirements for growth environment. It was first discovered in 1983 by Australian scientists in gastric mucosal biopsy specimens from patients with chronic active gastritis, and it is currently the only known microorganism capable of surviving in the human stomach [1]. Hp infection can cause diseases such as chronic gastritis and peptic ulcers, and persistent infection may also increase the risk of gastric cancer. Therefore, it has been listed as a Group 1 carcinogen by the

International Agency for Research on Cancer. Currently, the most commonly used clinical treatment for Hp is quadruple therapy. However, because long-term use of antibiotics easily leads to issues such as an increase in drug-resistant strains and imbalance of intestinal microbiota, seeking a natural, safe, and effective anti-Hp active ingredient has become a research direction. Sea cucumbers secrete a large amount of fucoidan into their body cavities as a defense mechanism to resist external invasion. Fucoidan is an important active polysaccharide extracted from sea cucumbers, possessing excellent antibacterial, anti-inflammatory, and immunomodulatory functions. Literature has reported that it can inhibit the growth and reproduction of some pathogenic bacteria. Our research group utilized in vitro cell experiments to screen fucoidan from sea cucumbers, aiming to discover a type of fucoidan with good anti-Hp effects and no toxic side effects on gastric mucosal epithelial cells, and to further explore the specific mechanisms of its efficacy, thereby laying a foundation for the development of new anti-Hp drugs.

1. Materials and Methods

1.1 Experimental Materials

Fresh sea cucumbers were sourced from a local seafood market and identified as Apostichopus japonicus. The Helicobacter pylori standard strain (ATCC 43504) was purchased from the China Center for Type Culture Collection (CCTCC). The Hp clinical drug-resistant strain was isolated from gastric mucosal biopsy samples of Hp-positive patients by the Department of Gastroenterology of our hospital (its identity was verified through urease tests, Giemsa staining, and 16S rDNA sequencing). Human gastric mucosal epithelial cells (GES-1) were purchased from the Cell Bank of the Chinese Academy of Sciences. RPMI 1640 medium, fetal bovine serum (FBS), and trypsin were purchased from Gibco. The CCK-8 kit and Giemsa staining solution were purchased from Beyotime Biotechnology Co., Ltd. IL-1β and TNF-α ELISA kits were purchased from Wuhan Boster Biological Engineering Co., Ltd. Other reagents were of analytical grade. Experimental equipment included a clean bench, a constant temperature incubator, an inverted microscope, a microplate reader, and a high-speed centrifuge.

1.2 Experimental Methods

Extraction and purification of sea cucumber fucoidan: Fresh sea cucumbers were selected, eviscerated, cleaned, and cut into small pieces. Ten volumes of distilled water were added, and extraction was performed in a 60°C water bath for 2 hours, repeated three times. The extracts were mixed and centrifuged at 3000 r/min for 15 minutes to remove precipitates. The supernatant was concentrated to approximately 1/5 of the original volume under vacuum, followed by the addition of 4 volumes of anhydrous ethanol and allowed to stand overnight at 4°C. The solid matter was then separated by centrifugation, resuspended in distilled water, desalted by dialysis (molecular weight cut-off of 6000 Da), and lyophilized to obtain the crude sea cucumber fucoidan. Further purification was performed using a Sephadex G-75 gel column to obtain high-purity sea cucumber fucoidan. The polysaccharide content was determined by the phenol-sulfuric acid method and set aside for use.

Hp culture and activation: The Hp standard strain and clinically isolated drug-resistant strain were inoculated onto

Columbia agar medium containing 5% sheep blood and cultured for 72 hours at 37°C under 5% CO_2 and microaerophilic conditions. Representative colonies were selected and transferred to Brucella broth. After shaking culture for 24 hours, the bacterial concentration was adjusted to 1×10^8 CFU/mL and set aside.

Determination of MIC and MBC: Using the micro-dilution method, sea cucumber fucoidan was diluted with Brucella broth into different concentrations (4.5, 9, 18, 36, 72, 144 mg/mL) and added to a 96-well plate (100 μL per well). Then, 100 μL of Hp bacterial suspension adjusted to the corresponding concentration was added to achieve a final concentration of 5×10^7 CFU/mL. Additionally, a negative control group (medium only) and a positive control group (medium and bacterial suspension) were established. After culturing for 72 hours under 37°C microaerophilic conditions, each well was observed for turbidity. The lowest polysaccharide concentration corresponding to non-turbid wells was defined as the MIC value. The bacterial suspension from non-turbid wells was inoculated onto Columbia blood agar plates and cultured for 72 hours. The lowest polysaccharide concentration at which no colonies appeared was defined as the MBC value. Three replicate wells were set for each concentration, and the experiment was repeated three times.

Cell culture and grouping: GES-1 cells were inoculated in RPMI 1640 culture medium containing 10% FBS and cultured in a 37°C, 5% CO_2 incubator. When cell growth reached over 80%, they were passaged using trypsin digestion. The experiment was divided into four groups: a blank control group (no Hp infection, no sea cucumber fucoidan added), an Hp infection group (Hp infection present, no sea cucumber fucoidan added), a low-dose sea cucumber fucoidan group (Hp infection present, 18 mg/mL sea cucumber fucoidan added), and a high-dose sea cucumber fucoidan group (Hp infection present, 36 mg/mL sea cucumber fucoidan added). Three replicate wells were set up for each group and the experiment was repeated three times [2].

Cell survival rate detection: GES-1 cells were inoculated into a 96-well plate. After incubating for 24 hours, the cells were treated according to the corresponding groups and incubated for another 24 hours. 10 μL of CCK-8 solution was added to each well and incubated at 37°C for 2 hours. The absorbance (OD value) was read at 450 nm using a microplate reader to calculate the cell survival rate. Cell survival rate (%) = [(OD value of the experimental group - OD value of the blank control group) / (OD value of the normal control group - OD value of the blank control group)] × 100% [3].

Cell morphology observation: GES-1 cells were inoculated onto 6-well plates and cultured for 24 hours. After treatment according to the grouping and another 24 hours of culture, the culture medium was aspirated, and cells were washed three times with PBS. Giemsa staining solution was added for 15 minutes. After washing away excess dye with distilled water, cell changes were observed under an inverted microscope and photos were saved.

Detection of inflammatory cytokines: After culturing cells under different treatment conditions, the culture supernatant was collected and centrifuged (3000 r/min, 10 min) to remove precipitates. Following the instructions of the ELISA kits, the levels of IL-1β and TNF-α in the supernatant were measured. The OD value was read at 450 nm using a microplate reader, and the concentration of inflammatory cytokines was derived from a standard curve.

Statistical processing: Data analysis was conducted using SPSS 22.0 software. Measurement data were expressed as mean ± standard deviation (x ± s). One-way analysis of variance was used for comparisons among multiple groups, and the LSD-t test was used for pairwise comparisons. A P value < 0.05 was considered statistically significant.

2. Results

2.1 In vitro inhibitory effect of sea cucumber fucoidan on Hp

The results of the microdilution assay indicated that the sea cucumber algal polysaccharide exhibited a good antibacterial effect against both the standard strain of Hp and the clinically isolated drug-resistant strains of Hp, with a concentration-dependent pattern. Specifically: for the standard Hp strain, its MIC value was 18 mg/mL and its MBC value was 36 mg/mL; while for the clinically isolated Hp strains, its MIC value was 36 mg/mL and its MBC value was 72 mg/mL. The blank control showed no turbidity, but the bacterial liquid control showed severe turbidity, indicating that the sea cucumber algal polysaccharide can inhibit the growth of Hp and is also effective for the clinically isolated strains. This is consistent with the relevant literature previously reported on the effective dosage range of sea cucumber algal polysaccharide for in vitro antibacterial activity against Hp [4]. The specific inhibition effect data are shown in Table 1.

Table 1 In vitro inhibitory activity of sea cucumber fucoidan against different Hp strains (n=3, x±s)

Strain Type	MIC, mg/mL	MBC, mg/mL	MBC/MIC
Standard Strain ATCC 43504	18	36	2
Clinical Drug-Resistant Strain	36	72	2

2.2 Effect of sea cucumber fucoidan on the survival rate of GES-1 cells after Hp infection

CCK-8 detection results showed that Hp infection caused damage to GES-1 cells, while sea cucumber fucoidan could alleviate this damage and promote cell survival; the higher the concentration, the stronger the protective effect. The specific cell survival rate data are shown in Table 2.

Table 2 Effect of sea cucumber fucoidan on the survival rate of GES-1 cells after Hp infection (n=3, x±s)

Experimental Group	Cell Survival Rate, %, x±s	Compared with Blank Control, P value	Compared with Hp Infection Group, P value
Blank Control	98.62±2.15	-	-
Hp Infection Group	52.37±3.89	<0.05	-
Low-concentration, 18mg/mL	75.83±4.26	<0.05	<0.05
High-concentration, 36mg/mL	88.95±3.57	>0.05	<0.05

2.3 Effect of sea cucumber fucoidan on the morphology of GES-1 cells after Hp infection

Observation under inverted microscope showed that in the blank control group, GES-1 cells had intact morphology, being mostly polygonal, closely arranged, well connected, and vigorous in growth; in the Hp infection group, the cell morphology changed significantly, becoming wrinkled and degenerated, taking on a round or irregular shape, with loose connections and some detachment, and a smaller number; in the low-dose sea cucumber rock algae polysaccharide group, the cell wrinkling and deformation were weakened, connections improved, and the number of detached cells decreased; while in the high-dose group, the cell morphology was similar to that of the blank

control group, closely arranged, with only a small amount of slight wrinkling and no significant detachment phenomenon. This indicates that sea cucumber rock algae polysaccharide can alleviate the morphological damage of GES-1 cells caused by Hp and there exists a certain dose-effect relationship.

2.4 Effect of sea cucumber fucoidan on inflammatory cytokine secretion of GES-1 cells after Hp infection

The ELISA test results indicate that Hp infection leads to the extensive production of inflammatory factors in GES-1 cells. At the same time, the sea cucumber algal polysaccharide can inhibit the generation of these inflammatory factors and reduce the degree of cell inflammation. This also conforms to the effect of sea cucumber algal polysaccharide in combating gastric inflammation caused by Hp through influencing inflammatory factors. The specific concentration data of inflammatory factors are shown in Table 3.

Table 3 Effect of sea cucumber fucoidan on inflammatory cytokine secretion of GES-1 cells after Hp infection (n=3, x±s)

ExperimentalGroup	IL-1βConcentration（pg/mL,x±s）	TNF-αConcentration（pg/mL,x±s）
Blank Control	12.35±1.87	18.62±2.34
Hp Infection Group	68.92±5.73	89.45±6.82
Low-concentration, 18mg/mL	42.56±4.18	56.73±5.29
High-concentration, 36mg/mL	25.87±3.64	32.45±4.08

3. Conclusion

In conclusion, this study found that the sea cucumber algal polysaccharide has a strong inhibitory effect on the standard strain of Hp as well as various Hp drug-resistant strains isolated from clinical cases. It can promote the recovery of gastric mucosal epithelial cell vitality after Hp infection, repair the damaged state of cells, reduce the production of inflammatory mediators such as IL-1β and TNF-α in cells, alleviate cellular inflammatory stress, and there is a concentration correlation. This indicates that the sea cucumber algal polysaccharide has good in vitro antibacterial ability and gastric mucosal cell protection effect. Its efficacy may be due to directly inhibiting the growth and reproduction of Hp and regulating the inflammatory signal transduction pathway. This provides a new direction for the development of natural antibacterial agents and lays a good foundation for further research. The sea cucumber algal polysaccharide is promising to become a new type of antibacterial agent for the treatment of Hp infection.

References

[1]李宝山.岩藻多糖、岩藻糖和葡萄糖对刺参生长、消化生理与糖代谢的影响[D].上海海洋大学,2024.

[2]陈倩妮.强免疫增强活性岩藻多糖的优化制备及构效分析[D].华南理工大学,2024.

[3]叶倩桃.海参岩藻糖基化硫酸软骨素对帕金森病小鼠模型的防治作用及机制研究[D].暨南大学,2022.

[4]张彤彤.海参多糖的测定方法优化及其体内代谢研究[D].大连工业大学,2021.