



Evaluating toxic effects of exopolysaccharide from a *Vibrio species* on human gastric and colon carcinoma cells *in vitro*

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Abstract

Gastric and colon carcinomas are among the most prevalent cancers with high mortality rates worldwide, and therefore, introduction of new anticancer agents has been widely considered for their treatment. The aim of present study was to determine cytotoxicity of exopolysaccharide (EPS) derived from a *Vibrio species* on human gastric and colon cancer cells. To do so, after isolation of EPS from a *Vibrio* strain VB23, MKN45 and LoVo cells were treated with 25, 50, 100 and 200 µg/ml of VB23 EPS. Upon 24 and 48 h, cell viability was evaluated by resazurin as a colorimetric assay. Results of viability assay indicated that 200 µg/ml of VB23 EPS induced highest toxic effects on MKN45 cells after 24 h. However, 100 µg/ml VB23 EPS induced highest cytotoxicity on LoVo cells after 24 h. Accordingly, obtained results revealed that cytotoxic effects of VB23 EPS were time- and cell type-dependent.

Keywords: *Vibrio*, exopolysaccharide, Gastric carcinoma, Colon carcinoma, Toxic effects.

Introduction

According to the latest reports on global cancer statistics, there were about 4.8 million new cases of gastrointestinal cancers and 3.4 million related deaths in 2018 [1]. Gastric adenocarcinoma (GA) represents one of the most common causes of cancer death worldwide. Systemic chemotherapy, radiotherapy, surgery, immunotherapy, and targeted therapy all have proven efficacy in the treatment of GA patients [2]. Colon carcinoma is also among the most lethal and prevalent malignancies in the world. Surgery, chemotherapy and radiotherapy have long been the first choices for colon cancer, although unsatisfied prognosis of the disease limits clinical outcomes [3]. Due to low survival rate of gastric and colon carcinomas, the introduction of new chemical or biological agents with more toxic and specific effects has been widely considered.

Bacterial exopolysaccharides (EPS) are organic macromolecules formed by polymerization of sugar and nonsugar components. Besides playing important roles in the interaction between



bacteria and their environment, they possess valuable properties such as antibacterial, antioxidative, antidiabetic and anticancer effects [4]. In present research, we investigated cytotoxic effects of EPS derived from a *Vibrio species* on human gastric and colon carcinoma cells.

Experimental

To obtain the bacterial isolates, serial dilution plating on thiosulfate-citrate-bile salts-sucrose agar and glycerol-based marine agar plates were incubated at 28°C for 24 h. Upon isolation, the exopolymer-producing bacteria were screened for their ability to produce exopolymer based on colony morphology (mucoid phenotypes). Among the screened isolates, *Vibrio* strain VB23 with the ability to form a viscous exopolymer was selected. To produce EPS, VB23 was grown in optimized mineral salts medium containing 0.2% glucose, 12.6% K₂HPO₄, 18.2% KH₂PO₄, 10% NH₄NO₃, 1% MgSO₄·7H₂O, 0.6% MnSO₄, 1% sodium molybdate, 1% CaCl₂·2H₂O, 0.06% FeSO₄·2H₂O and 1.5% of NaCl in 1000 mL distilled H₂O. For extraction and purification of VB23 EPS, the culture was centrifuged, supernatants were precipitated by adding three volumes of cold ethanol and solution was chilled at 4°C overnight and lyophilized.

Gastric adenocarcinoma cells, MKN45 cell line, were cultured in Dulbecco's modified Eagle's medium (Biowest), while colon carcinoma cells, LoVo cell line, were grown in Roswell Park Memorial Institute -1640 (Capricorn), both supplemented with 10% fetal bovine serum (Biowest) and 1% penicillin-streptomycin (Biowest). Human cells were incubated at 37°C and 5% CO₂ in air, and subcultured by 0.25 % trypsin-1 mM EDTA (Betacell). To assess toxicity of VB23 EPS, MKN45 and LoVo cells were seeded in 96 well plates with densities of 10000 and 13000 cells in each well, respectively. After 24 h, cells were treated 25, 50, 100 and 200 µg/ml of VB23 EPS for 24 and 48 h. Then, cell viability was evaluated by resazurin (Sigma) as a colorimetric assay. To do so, resazurin (20 µl/well) was added to treated cells, as well as their untreated controls, and after incubation at 37°C for 2 h, the optical density (OD) of each cells was measured at 600 nm. To calculate cell viability (%), the following formula was used: $100 \times (100 - (T_{OD} - U_{OD}) / (B_{OD} - U_{OD}))$, in which T_{OD}, U_{OD} and B_{OD} were OD of treated cells, untreated cells and blank control, respectively.

Results and discussion

Viability assessment of MKN45 cells (Figure 1) revealed that upon 24 h treatment with 25, 50, 100 and 200 µg/ml VB23 EPS, 87%, 90%, 88% and 72% of cells were viable, respectively. In addition, quantitative analysis of cell viability showed that 93%, 92%, 96% and 88% of cells were alive after 48 h treatment with 25, 50, 100 and 200 µg/ml VB23 EPS, respectively. Accordingly, the highest concentration of VB23 EPS induced highest toxic effects on MKN45 cells after 24 h.

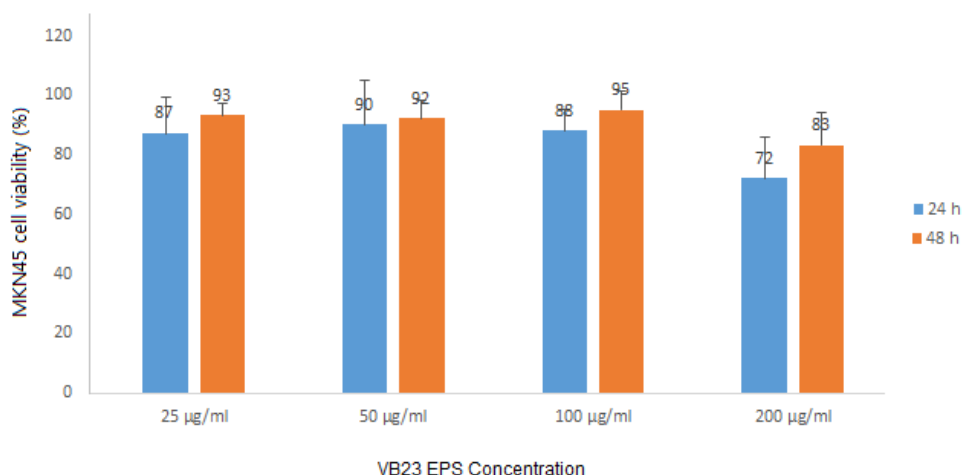


Figure 1. Viability assessment of GA cells (MKN45 cell line) after 24 and 48 h treatment with increasing concentrations of VB23 EPS.

As shown in Figure 2, viability of LoVo cells was calculated as 82%, 65%, 61% and 73% after 24 h treatment with 25, 50, 100 and 200 µg/ml VB23 EPS, respectively. Moreover, 82%, 88%, 86% and 86% of cells were viable upon 48 h treatment with 25, 50, 100 and 200 µg/ml VB23 EPS, respectively. Based on obtained results, 100 µg/ml VB23 EPS induced highest toxic effects on LoVo cells after 24 h.

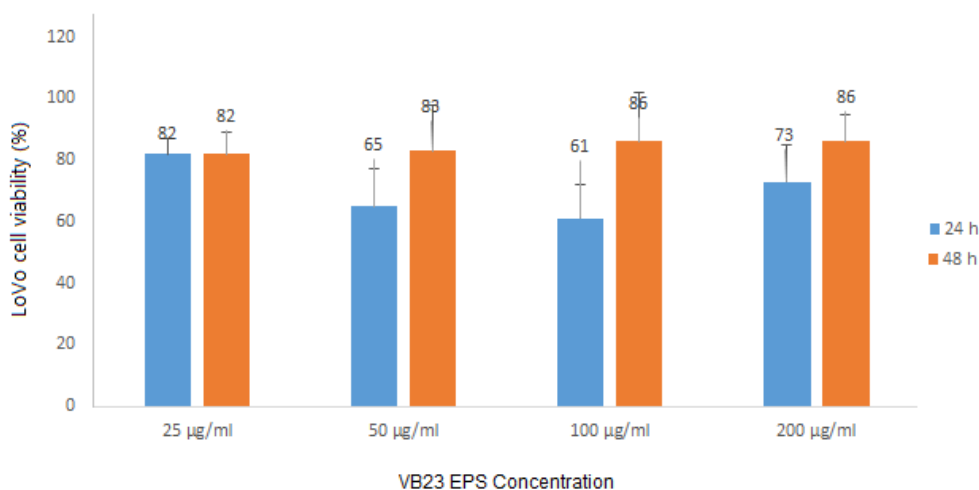


Figure 2. Viability assessment of colon cancer cells (LoVo cell line) after 24 and 48 h treatment with increasing concentrations of VB23 EPS.

Conclusions

Results of present research indicated that cytotoxic effects of VB23 EPS were time- and cell type-dependent; lowest viability of MKN45 and LoVo cells were determined after 24 h treatment with 200 and 100 µg/ml VB23 EPS, respectively, and increasing the treatment period up to 48 h did not increase cytotoxicity.



Acknowledgements

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