ACTIVITY OF OLEIC ACID ON BIOFILM FORMATION OF *S. aureus*

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Oleic acid is a naturally occurring fatty acid in animal and vegetable oils and has been shown to have a wide variety of pharmacological effects. It aimed to investigate the efficacy of oleic acid on the adhesion and invasion of *S. aureus* to the host cell and biofilm production. The standard *S. aureus* strain (ATCC 25923) was used in the experiments. Cytotoxicity tests of oleic acid were performed in the Vero cell line. Bacterial adhesion and invasion rates and activities on slime formation in cells treated with oleic acid were evaluated compared to the control group. Slime formation tests were evaluated phenotypically on Congo red agar. In the study, it was determined that oleic acid was effective in both cellular adhesion and invasion in terms of colony number in cell cultures treated with 0.156 µg/ml concentration of oleic acid. In addition, it was determined that slime production was significantly inhibited in bacterial cultures treated with oleic acid. Oleic acid prevents cells from attaching to bacteria and has an inhibitory effect on the virulence of bacteria. This activity of oleic acid may be due to its modulatory effect on cellular processes, and its bacterial virulence may be related to its effect on bacterial metabolism.

Keywords: oleic acid, adhesion, invasion, biofilm

INTRODUCTION

Oleic acid is a monounsaturated fatty acid. It is found in nature in vegetable and animal oils. Oleic acid is the most abundant fatty acid in human adipose tissue and is second only to palmitic acid in human tissues overall. Oleic acid is an unsaturated fatty acid found as a glyceryl ester in various vegetable oils such as hazelnut and olive oil (Sales-Campos *et al*., 2013; Carrillo *et al*., 2012; Carrillo *et al*., 2012).

Oleic acid constitutes 500-85% of the total fatty acids that form a triglyceride complex in olive oil. Scientific studies have shown that oleic acid and its derivatives exhibit a variety of biological activities, including antimicrobial and anticancer activities. In the literature, it has been reported that oleic acid and extracts containing oleic acid have various biological effects such as antibacterial, antifungal, antiviral, and antioxidant activity (Sales-Campos *et al*., 2013; Carrillo *et al*., 2012). Although rich pharmacological activities of oleic acid have been reported, studies on its effectiveness in host cell adhesion and invasion and its effects on bacterial virulence are limited.
In this study, the activities of oleic acid on the cultured cells and the biofilm formation of *S. aureus* were evaluated.

Bacterial Strain

Standard *Staphylococcus aureus* (ATCC 25923) strain from the bacterial culture collection of Hatay Mustafa Kemal University Faculty of Medicine, Department of Medical Microbiology was used in the experiments.

Strains, Cell Culture, and Oleic Acid

The Vero cell line, which was used for both cytotoxicity tests and in-vitro efficacy tests in the study, was obtained from the culture collection of Hatay Mustafa Kemal University Faculty of Medicine, Department of Medical Microbiology, and oleic acid was obtained commercially.

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In the study, RPMI 1640 broth containing 10% fetal calf serum, 10 mM HEPES, and 100 IU/ml penicillin/streptomycin with 4 mM glutamine was used for cell growth and maintenance in cell culture experiments.

Incubation of cells was carried out in an incubator at 37 °C, 5% CO_2, and 95% air. For the production of cells, they were incubated in culture dishes of different volumes (100, 250, and 500 ml) as $1x10^6$ cells/ml, in containers containing 10 percent of the culture vessel. For the proliferation of the cells, the growth was followed daily for 3 days, and passage procedures were performed when the cells were grown in a monolayer manner on the surface of the culture dish. The cells were removed from the culture dish with the prepared trypsinization solution and transferred to 50 ml centrifuge tubes at 1500 rpm for 15 minutes. The cell pellet was collected by centrifugation.

Activity assays were performed on flat-bottomed microplates. Dimethyl sulfoxide (DMSO) used to dissolve oleic acid was used. Tests for the determination of the non-toxic concentration of DMSO were performed using the Vero cell line. The non-toxic concentration of DMSO was chosen as the solvent concentration.

Activity Studies

Firstly, non-toxic concentrations of oleic acid in Vero cell culture were determined. Activity studies were performed within these non-toxic concentrations. Studies to determine the cytotoxic effect were performed with the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method as described by Mossman (Mossman *et al*., 1983). For activity studies, cell cultures were prepared with $1x10^6$ cells in each ml of Vero cells. After 6 hours of incubation for cell adhesion, amounts of chemical compounds containing different concentrations were added to the culture medium.

After incubation, the cells that continued to proliferate by adhering to the culture dish surface were treated with 0.25% trypsinization solution and transferred to centrifuge tubes. Cells were collected by centrifugation at 1500 rpm for 10 minutes in a refrigerated centrifuge (+4 °C) and cell viability was determined.

Cell Adhesion Tests

Fresh cultures of *S. aureus* passaged on Mueller-Hinton agar were used for this purpose. Before these procedures, the Vero cell line was prepared with 1x106 cells in each ml. Then, Oleic acid (different concentrations (of 5, 10, 15, 20, and 25 µg/ml) was added to the cells. After inoculation, they were incubated at 37 °C for 2 hours.

Then, a $1x10^8$ concentration of bacteria solution prepared from 24-hour fresh cultures of *S. aureus* was added to cell culture media treated with oleic acid. Adhesion tests were performed by incubating at 37 °C for 3 hours. The wells were washed with PBS solution. This procedure was repeated 3 times. The cells were then treated with 0.025% Triton X-100 and incubated with this solution for 5 minutes at room temperature. At the end of the incubation, the samples taken from the wells were inoculated into Mueller-Hinton agar and a bacterial count was performed.

Invasion Tests

For invasion tests, after bacterial adhesion to the cells, the cell surface was washed with an antibiotic solution (gentamicin solution; 200 µg/mL). Cells with an antibiotic solution at 37 °C for 15 min. incubated. Thus, bacteria on the cell surface were

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inactivated. Then, the wells were washed 3 times with PBS solution. Then, 0.025% Triton X-100 was added to the cells and incubated for 5 minutes at room temperature. The lysate formed in the wells was homogenized and the sample taken from each well was inoculated with Mueller-Hinton agar for the bacterial count and incubated (24 hours at 37 °C).

Slime Production

The presence of the effect of oleic acid on the slime production of *S. aureus* was evaluated by the Congo-red method, one of the phenotypic methods.

Slime production from bacteria exposed to these chemical compounds and different combinations of these compounds was compared with slime-positive Saureus strains by inoculation on a Congo-red medium. In the evaluation made after a 48-hour incubation at 37 °C following the microorganism inoculation, the presence of black colonies in Congo-red medium was evaluated as slime production positive, and the presence of light-colored colonies was evaluated as negative slime production.

Results

Oleic acid exhibited döşe-dependent activity on the adhesion of *S. aureus* to Vero cells. Compared to the control group, it was determined that there was no effect on cell adhesion in the presence of 5 µg/ml oleic acid, but bacterial adhesion was inhibited statistically in the presence of 10, 15, 20, and 25 µg/ml oleic acid (Figure 1).

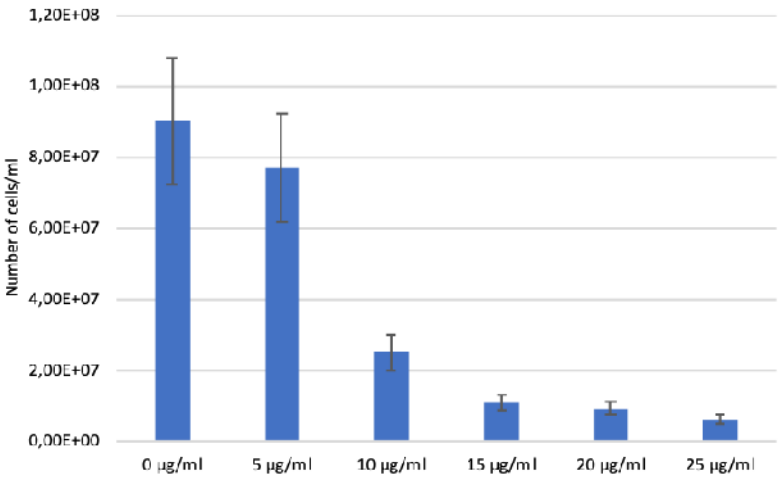

Figure 1. Efficacy of *S. aureus* adhesion to Vero cells at different concentrations of oleic acid

A similar relationship was also obtained in the invasion tests performed following the adhesion experiments. Unlike the adhesion tests, oleic acid significantly inhibited the invasion of *S. aureus* into the host cell at 5 µg/ml and all other concentrations (Figure 2).

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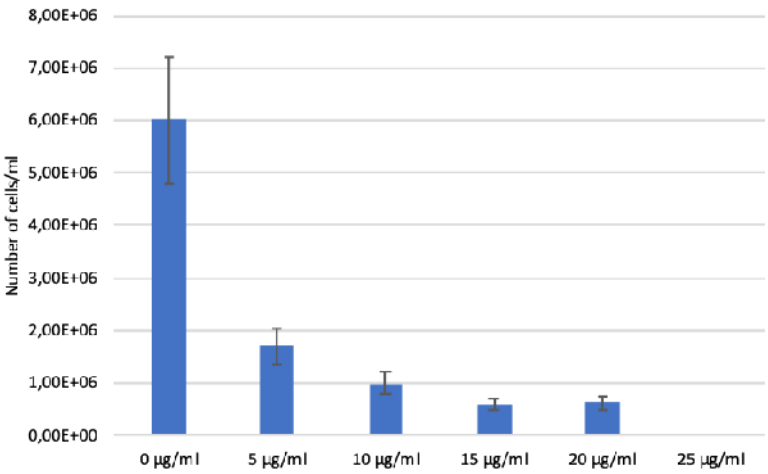

Figure 2. Efficacy of *S. aureus* invasion into Vero cells at different concentrations of oleic acid

The efficacy results of oleic acid on the biofilm-forming capacity of S.aureus are given in Figure 3. As can be seen from the figure, although oleic acid produced a reduction in bacterial biofilm formation at 5 and 10 µg/ml concentrations, this inhibition was not significant when compared to the control group. However, oleic acid significantly inhibited biofilm formation at concentrations of 15, 20, and 25 µg/ml (Figure 3).

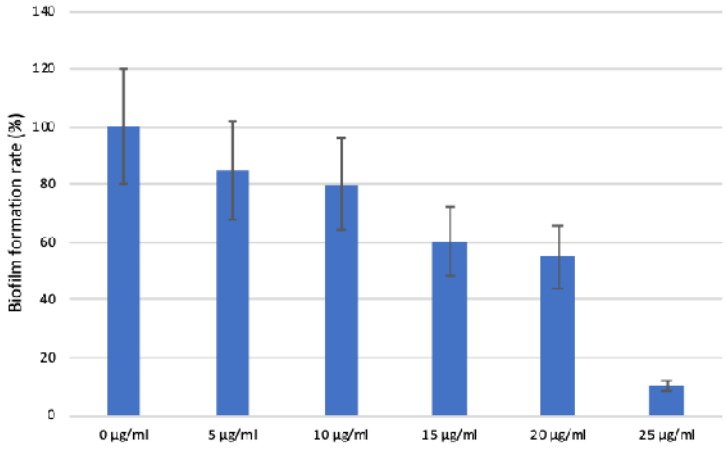

Figure 3. The concentration-related activity of oleic acid on biofilm formation of *S. aureus*

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DISCUSSION

In recent years, it has been reported in various studies that oleic acid has very important contributions to human health and diseases (Ghavam *et al*., 2021; Yamagata *et al*., 2021; Li *et al*., 2014). In our study, it was determined that when the intracellular oleic acid level is high, both the cell resistance of the host cell against the pathogen increases and the biofilm formation capacity of *S. aureus*, which is one of the most important virulence factors, is inhibited.

CONCLUSIONS

The fact that oleic acid makes the host cell resistant to bacterial adhesion and invasion can be considered an indicator of its being a cellular modulator. These results will be very valuable in the treatment of virulent *S. aureus* strains. The combined use of oleic acid with any antibiotic can increase drug efficacy by inhibiting biofilm formation.

REFERENCES

Carrillo, C., Cavia, M.ª del M. and Alonso-Torre, S.R. (2012), "Antitumor Effect of Oleic Acid; Mechanisms of Action: A Review", *Nutrición Hospitalaria*, 27(6), 1860-5, https://doi.org/10.3305/nh.2012.27.6.6010.

Carrillo, C., Cavia, M.ª del M. and Alonso-Torre, S. (2012), "Role of Oleic Acid in the Immune System; Mechanism of Action: A Review", *Nutrición Hospitalaria*, 27(4), 978-990, https://doi.org/10.3305/nh.2012.27.4.5783.

Ghavam, M., Afzali, A. and Manca, M.L. (2021), "Chemotype of Damask Rose with Oleic Acid (9 Octadecenoic Acids) and Its Antimicrobial Effectiveness", *Scientific Reports*, 11(1), 8027, https://doi.org/10.1038/s41598-021-87604-1.

Li, S., Zhou, T., Li, C., Dai, Z., Che, D., Yao, Y., Li, L., Ma, J., Yang, X. and Gao, G. (2014), "High Metastatic Gastric and Breast Cancer Cells Consume Oleic Acid in an AMPK Dependent Manner", *PLoS One*, 9(5), e97330, https://doi.org/10.1371/journal.pone.0097330.

Mosmann, T. (1983), "Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays", *Journal of Immunological Methods*, 65(1-2), 55-63, https://doi.org/10.1016/0022-1759(83)90303-4.

Sales-Campos, H., Souza, P.R., Peghini, B.C., da Silva, J.S. and Cardoso, C.R. (2013), "An Overview of the Modulatory Effects of Oleic Acid in Health and Disease", *Mini-Reviews in Medicinal Chemistry*, 13(2), 201-210, https://doi.org/10.2174/1389557511313020003.

Yamagata, K., Uzu, E., Yoshida, Y., Kato, C. and Tagami, M. (2021), "Oleic Acid and Oleoylethanolamide Decrease Interferon-γ-Induced Expression of PD-L1 and Induce Apoptosis in Human Lung Carcinoma Cells", *European Journal of Pharmacology*, 903, 174116, https://doi.org/10.1016/j.ejphar.2021.174116.

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