EFFICACY OF CAPSAICIN ON CELL ADHESION AND INVASION OF ORAL PATHOGENS

ERHAN TEK[1], NIZAMI DURAN[2]

[1]*Mustafa Kemal University, Veterinary Faculty, Microbiology Department, Hatay-Türkiye, etek@mku.edu.tr*

[2]*Mustafa Kemal University, Medical Faculty, Microbiology Department, Hatay-Türkiye, nizamduran@hotmail.com*

Streptococcus pyogenes, *Streptococcus mutans*, and *Candida albicans* are important human pathogens and their infections in the mouth, mouth, and throat are important. Prophylaxis against oral and respiratory tract infections is of great importance in terms of both reducing the use of antibiotics and lowering the infection frequency. This study investigated the antimicrobial activity of Capsaicin against *S. mutans*, *C. albicans*, and *S. pyogenes*. Non-cytotoxic concentration of Capsaicin was determined in the Vero cell line by the MTT method. Efficacy studies were performed within these determined non-cytotoxic concentrations. The efficacy of single and different combinations of these three biological components on cell adhesion and invasion. The non-toxic concentration of capsaicin on Vero cells was <1.35 µg/ml. Capsaicin exhibited significant antimicrobial activity against *S. pyogenes*, *S. mutans*, and *C. albicans*. Moreover, capsaicin was statistically significantly effective against host cell adhesion and invasion against *S. mutans*, *S. pyogenes* and *C. albicans* compared to the control group. The results showed that capsaicin is a highly potent antibacterial agent against *S. pyogenes*, and *S. mutans*, as well as an important prophylactic agent for fungal infections. As a result, we think that capsaicin is a useful molecule for the provision and maintenance of both respiratory diseases and oral health.

Keywords: *S. pyogenes*, *S. aureus*, *C. albicans*, throat infection, proliaxia, lozenge

INTRODUCTION

A wide variety of microorganisms can cause infections in the human oral cavity. The mouth is a unique region colonized by bacteria, fungi, viruses, and even protozoa. Throat infections are mainly caused by viruses, and bacterial and fungal pathogens are also important infectious agents for the oral region (Zhang *et al*., 2018).

Among the pathogens that cause throat infections, *Streptococci*, *staphylococci*, and *Candida* are among the most important pathogens that come to mind first (Vlastarakos *et al*., 2007). *Streptococcus pyogenes* can cause late complications of rheumatic fever and glomerulonephritis. In addition, it is one of the most frequently detected bacterial agents of pharyngitis and skin infections. *Streptococcus pyogenes* is one of the leading pathogenic bacteria infecting children and adolescents and can cause a wide variety of infections. It is reported that more than 600 million throat infections are caused by *S. pyogenes* in the world annually (Fiedler *et al*., 2015; Walker *et al*., 2014).

Dental caries is one of the most common infectious diseases in the world. It is a serious threat to oral and dental health. One of the main pathogens that play an important role in the development of dental caries is *Streptococcus mutans*. *S. mutans* is an acidogenic and acidic Gram-positive bacterium that naturally lives in the oral cavity. The natural habitat of *S. mutans* is the human oral cavity, more specifically dental plaque, a multi-species biofilm that forms on the hard surfaces of the tooth (Krzyściak *et al*., 2014; Struzycka, 2014).

Candida can cause serious infections, especially in children, the elderly, and immunocompromised hosts. *Candida albicans* is a pathogenic yeast that causes oral, vaginal, and systemic infections. In recent years, it has been reported that there is a

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significant increase in the frequency of *C. albicans* infections due to the prolonged human lifespan and the increase in immunosuppressed hosts (Silva *et al*., 2012; Poulain, 2015). In this study, we found that capsaicin was effective against *Streptococcus pyogenes*, *S. mutans*, and *C. albicans*.

MATERIALS AND METHODS

Strains Used in the Study

Vero cell line, *Streptococcus pyogenes*, *Streptococcus mutans*, and *Candida albicans* strains were obtained from the culture collection of Hatay Mustafa Kemal University Faculty of Medicine, Department of Medical Microbiology and Capsaicin was obtained commercially.

Cell Culture

The cytotoxicity and invasion and adhesion tests were performed on the Vero cell line. In cell culture experiments, 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. Incubation of cells was carried out in an incubator at 37°C, 5% CO_2, and 95% air. For the production of cells, it was carried out in culture dishes of different volumes (100, 250, and 500 ml) as $1x10^6$ cells/ml. When the cells were grown as a monolayer on the culture dish surface, the passage of the cells was carried out. The cells were removed from the culture dish with trypsinization solution and transferred to 50 ml centrifuge tubes, at 1500 rpm for 15 min. collected by centrifugation. Cell viability and number were determined under the microscope in a hemocytometer with 1% trypan blue dye prepared in 0.9% NaCl.

Activity assays were performed in 96-well flat-bottom microplates. Cells were inoculated into the wells with RPMI 1640 medium containing 10% fetal calf serum at $1x10^6$ per ml. In the experiments, dimethyl sulfoxide (DMSO) (Sigma, MI, USA) was used to dissolve Capsaicin.

Cell Viability Determination

Cell viability assay was performed with trypan blue dye. The basis of staining method is based on staining dead cells exposed to Trypan blue dye due to the deterioration of their membrane integrity. Cells removed by trypsinization during both the passage of Vero cells and activity studies were evaluated microscopically after treatment with trypan blue and incubated with dye at room temperature. Stained cells were considered dead, and unstained cells were considered alive. The mixture, which was incubated for 15 minutes at room temperature, was examined under a microscope to determine viability. Cell count was performed with a hemocytometer.

ACTIVITY STUDIES

Preparation of Cell Culture

Firstly, non-toxic concentrations of Capsaicin were determined in Vero cell culture. Activity studies were performed within these non-toxic concentrations. Studies

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to determine the cytotoxic effect were performed with the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method as described below. For activity studies, cell cultures were prepared with 1x106 cells in each ml of Vero cells. After 6 hours of incubation for cell adhesion, amounts of capsaicin containing different concentrations were added to the culture medium.

Cultures containing only the concentration of DMSO used as the solvent were used as the control group. Cultures without capsaicin were determined as the negative control group. All experiments were performed in triplicate, repeating 2 times. 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.

Efficacy of Capsaicin on Microorganism Reproduction in Vero Cell Line

For the adhesion of the cells, capsaicin was added to the cells incubated for 24 hours and incubated at 37 °C for 1 hour. Then, suspensions of microorganisms at a density of 1x106/ml were prepared from 24-hour cultures. These suspensions were transferred onto cell cultures in which capsaicin was inoculated and incubated at 37 °C for 3 hours. To determine the growth densities of microorganisms, both absorbance measurements and the samples taken from each well were inoculated into Mueller-Hinton agar and growth controls were carried out. Colony counts were calculated as CFU/ml. Thus, the activities of capsaicin on microorganisms were evaluated by quantitation.

Effects of Capsaicin on Cell Adhesion and Invasion of *S. pyogenes, S. mutans* and *C. albicans*

For this purpose, fresh cultures of microorganisms passaged on Mueller-Hinton and Saboraud Dextrose agar were used. Before these procedures, the Vero cell line was prepared with $1x10^6$ cells per ml. Capsaicin was inoculated into the cells at non-toxic concentrations on the cells that covered at least 80% of the culture dish surface. Following inoculation, the cultivation of cells was carried out at 37 °C for 2 hours. Then, microorganism cultures prepared from 24-hour cultures of microorganisms (for bacteria: $1x10^8$/ml; for C.albicans $1x10^6$/ml) were added to cell culture media treated with capsaicin. Incubation of cultures at 37 °C for 3 hours was carried out to finalize the adhesion experiments. PBS solution was added to the wells of the plate and aspirated gently. This process was repeated 3 times and the cells were washed. After this stage for adhesion tests; cells were treated with 0.025% Triton X-100 and incubated with this solution for at least 5 minutes at room temperature. At the end of the incubation, the mixture consisting of cells and microorganisms was homogenized and the samples taken from each well of the plate were inoculated into agar media. Bacterial colony counts were performed 24 hours after incubation.

Invasion Experiment

As mentioned above, Capsaicin treatment in cell cultures was added to the wells of the plate by preparing a solution of Gentamicin and Fluconazole for adhesion tests of bacteria to cells. After the addition of the antibiotic solution, the culture plates were incubated for a short time (approximately 10 minutes) at 37°C. During this time, the cell surface and microorganisms in the medium were inactivated. Then, PBS solution was

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added to the wells of the plate and the cell surfaces were washed 3 times as in the adhesion experiments above. After this step, 0.025% Triton X-100 was added to the cells and incubated for about 5 minutes at room temperature. The lysate formed in the wells was homogenized and the sample taken from each well was inoculated on Mueller-Hinton agar, and colony counts were carried out after 24 hours of incubation at 37°C.

RESULTS

The non-toxic concentration of capsaicin on Vero cells was <1.35 µg/ml. Capsaicin exhibited significant antimicrobial activity against *S. pyogenes*, *S. mutans*, and *C. albicans* (Figure 1).

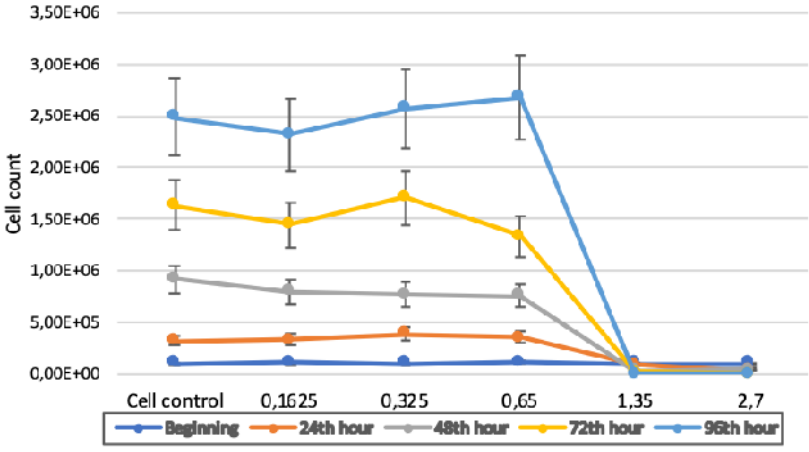

Figure 1. Determination of the non-toxic concentration of capsaicin in Vero cell culture

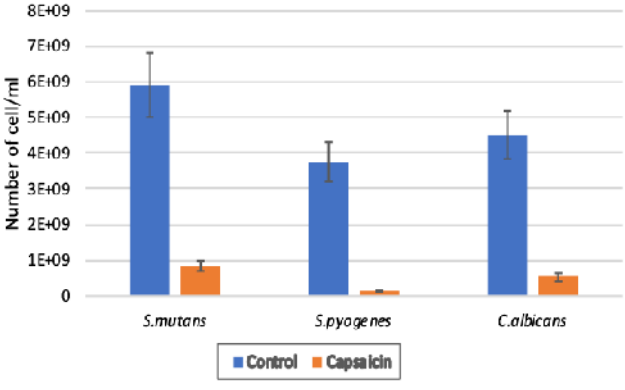

Figure 2. Antimicrobial activity of capsaicin against *S. mutans*, *S. pyogenes* and *C. albicans*

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As seen in Figure 2, capsaicin exhibited significant antimicrobial activity against *S. mutans*, *S. pyogenes*, and *C. albicans*. It was determined that there was a significant inhibition of growth against all three pathogens in the number of microorganisms.

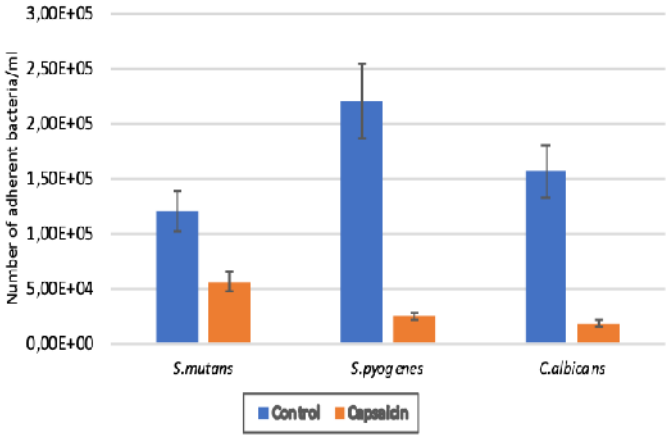

Figure 3. Effects of capsaicin on adhesion of microorganisms to Vero cells

As can be seen from Figure 3, Capsaicin inhibited the adhesion of microorganisms to Vero cells. This activity is very strong against *S. pyogenes* and *C. albicans*.

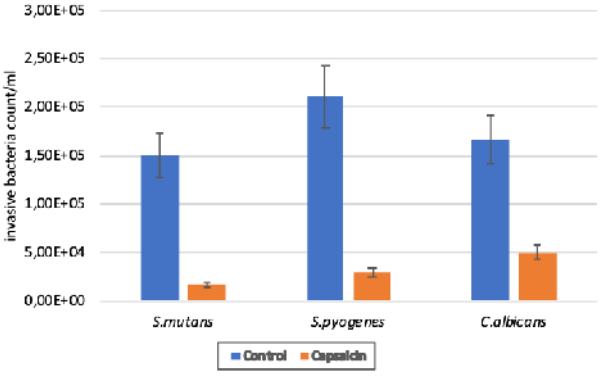

Figure 4. Effects of capsaicin on the invasion of microorganisms into Vero cells

It has been determined that capsaicin is very effective against cell invasion of cells that have made host cell adhesion (Figure 4).

CONCLUSION

Oral and dental diseases and respiratory tract infections are among the most common diseases in humans. *S. pyogenes*, *S. mutans*, and *C. albicans* can cause

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significant infections in the oral region. Today, various throat lozenges containing antiseptic, local anesthetic, and alcohol are used to relieve the symptoms of sore throat. Capsaicin showed very strong activity against *S. pyogenes*, *S. mutans*, and *C. albicans*, which are among the important oral pathogens. Capsaicin both significantly reduces the host cell adhesions of these pathogens and prevents the invasion of microorganisms with low adhesion into the cell. It is a potent agent both against oral and respiratory pathogens and against other human pathogens colonizing the host cell and causing inoculation for humans. The results should be verified with further studies. We believe that capsaicin can be used as a prophylaxis against such pathogens.

REFERENCES

Fiedler, T., Köller, T. and Kreikemeyer B. (2015), "*Streptococcus pyogenes* Biofilms-Formation, Biology, and Clinical Relevance", *Frontiers in Cellular and Infection Microbiology*, 5, 15, https://doi.org/10.3389/fcimb.2015.00015.

Krzyściak, W., Jurczak, A., Kościelniak, D., Bystrowska, B. and Skalniak, A. (2014), "The Virulence of *Streptococcus* Mutans and the Ability to Form Biofilms", *European Journal of Clinical Microbiology & Infectious Diseases*, 33(4), 499-515, https://doi.org/10.1007/s10096-013-1993-7.

Poulain, D. (2015), "*Candida albicans*, Plasticity, and Pathogenesis", *Critical Reviews in Microbiology*, 41(2), 208-217, https://doi.org/10.3109/1040841X.2013.813904.

Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D.W. and Azeredo, J. (2012), "*Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*: Biology, Epidemiology, Pathogenicity, and Antifungal Resistance", *FEMS Microbiology Reviews*, 36(2), 288-305, https://doi.org/10.1111/j.1574-6976.2011.00278.x.

Vlastarakos, P.V., Nikolopoulos, T.P., Maragoudakis, P., Tzagaroulakis, A. and Ferekidis, E. (2009), "Biofilms in Ear, Nose, and Throat Infections: How Important Are They?", *Laryngoscope*, 117(4), 668-673, https://doi.org/10.1097/MLG.0b013e318030e422.

Walker, M.J., Barnett, T.C., McArthur, J.D., Cole, J.N., Gillen, C.M., Henningham, A., Sriprakash, K.S., Sanderson-Smith, M.L. and Nizet, V. (2014), "Disease Manifestations and Pathogenic Mechanisms of Group A *Streptococcus*", *Clinical Microbiology Reviews*, 27(2), 264-301, https://doi.org/10.1128/CMR.00101-13.

Zhang, Y., Wang, X., Li, H., Ni, C., Du, Z. and Yan F. (2018), "Human Oral Microbiota and Its Modulation for Oral Health", *Biomedicine and Pharmacotherapy*, 99, 883-893, https://doi.org/10.1016/j.biopha.2018.01.146.

https://doi.org/10.24264/icams-2022.III.19