Research in Molecular Medicine

Short Research Article
2014, Vol: 2, Issue: 4, Pages: 33-35
DOI: 10.18869/acadpub.rmm.2.4.33

In Vitro Evaluation of Capsaicin Inhibitory Effects on Zonula Occludens Toxin in *Vibrio Cholerae* ATCC14035 Strain

Soroor Erfanimanesh [1], Gita Eslami [1*], Hossein Goudarzi [1], Arezou Taherpour [2], Ali Hashemi [1], Elahe Taki [1]

[1]Department of Microbiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Microbiology Department, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Received: 2 Aug 2014

Revised : 31 Aug 2014

Accepted: 19 Sep 2014

Corresponding Authors:
Gita Eslami
Department of Microbiology, Shahid Beheshti University of Medical Sciences, Koodakyar St., Tabnak Blv., Yaman Av.,Chamran highway, Tehran, Iran.
Phone: +98-2123872556
E-mail: g_eslami@yahoo.com

Abstract

Background: Cholera is one of the most potent diarrheal diseases that is characterized by massive watery stools, frequently accompanied by vomiting and can lead to hypovolemic shock and acidosis. It is caused by certain strains of *Vibrio cholerae* species which can also cause mild or in apparent infections. The aim of this study was to evaluate Capsaicin, as a potential inhibitor of zonula occludens toxin production in *V. cholerae* ATCC 14035.

Materials and Methods: MIC of capsaicin was determined by Broth Microdilution method according to CLSI guidelines. The *zot* gene expression level was analyzed using real-time RT–PCR.

Results: In MIC test, we found 100 $\mu g\ mL^{-1}$of capsaicin as the highest concentration that did not affect the bacterial growth; however, zonula occludens toxin (*zot*) gene expression of the tested strain was significantly inhibited by capsaicin in a dose-dependent manner at sub-bacteriocidal concentrations. The *rec*A gene did not show any significant difference in its expression with or without capsaicin.

Conclusion: Capsaicin is one of the active compounds of red chili that can drastically suppress *zot* gene expression and shows promising inhibitory effect against *V. cholerae zot* production. Thus, routine intake of red chili, which is easily available and inexpensive, may be an alternative approach to prevent and control symptoms of cholera.

Keywords: Capsaicin; Zonula Occludens Toxin (ZOT); *Vibrio Cholera*; Real-time PCR

Please cite this article as: Erfanimanesh S, Eslami G, Goudarzi H, Taherpour A, Hashemi A, Taki E. In Vitro Evaluation of Capsaicin Inhibitory Effects on Zonula Occludens Toxin in Vibrio Cholerae ATCC14035 Strain. Res Mol Med. 2014; 2 (4): 33-35

Introduction

Vibrio cholerae, the causative agent of the severe diarrheal disease cholera, is responsible for approximately 120,000 deaths annually (1, 2). The only known vertebrate host for this bacterium is human. After ingestion, most of the bacteria are killed by gastric acid. Persisting organisms colonize the small intestine that is the appropriate niche for this bacterium (2). Cholera still remains a major global health concern with numerous detected cases each year (3). The disease can spread by ingestion of contaminated food or water and is therefore related to poverty and inadequate sanitation (2). One of the most important virulence factors that play part in establishment of cholera symptoms is zonula occludens (ZOT). This toxin increases the permeability of ileal mucosa by affecting the structure of the intercellular tight junctions (3). Since the last couple of decades a large body of investigations has been allocated to medicinal properties of different natural products because of their potent pharmacological properties, convenience of use, low toxicity and economic viability (4). The uses of antibacterial agents are generally accepted as a key therapeutic target for bacterial diseases, however a large number of epidemic *V. cholerae* strains, have also become resistant to multiple antibacterial agents via mutations, horizontal gene transfer, etc. Antibacterial agents are commonly

bactericidal or bacteriostatic and accordingly have no effect on virulence gene expression. Since ancient times, natural products have been used to treat diarrheal diseases.

One of the active ingredients of red chili is capsaicin (*N*-anillyl-8-methyl-nonenamide), which can also act as an antibacterial agent against bacterial pathogens, for example *Bacillus* spp., *Helicobacter pylori*, etc. (5). Due to the alarming rise of antibiotic resistance there is a global call for new therapeutic approaches. Natural compounds have always been a product of interest because of their great safeness, fewer side effects, huge diversity and abundance of bioactive compounds that makes them an appropriate collection to examine for effective therapeutic options. In this study, we examined whether capsaicin can pose inhibitory effect on zonula occludens toxin (*zot*) gene expression in *V. cholerae*. ATCC 14035.

Materials and Methods

This was an Experimental study conducted on standard type strain of *V. cholerae* ATCC14035 classic biotype, serogroup O1, and serotype Ogawa.

Minimum Inhibitory Concentration (MIC)

MIC of capsaicin was determined by Broth Microdilution method according to CLSI guidelines (6). The standard strain was grown in MYEP medium at 37 °C up to the late logarithmic phase (~2 × 10^8 CFU mL^{-1}) with and without capsaicin (0µg mL^{-1}, 1 µg mL^{-1}, 10 µg mL^{-1}, 25 µg mL^{-1}, 50 µg mL^{-1} and 100 µg mL^{-1}).

RNA Extraction, cDNA synthesis and Real-Time PCR

Total RNA was extracted and purified using RNXTM-Plus Solution kit (Sinaclon, Iran) according to the manufacturer's instructions. The qRT-PCR assay was carried out by *Zot* gene-specific primers. A housekeeping *recA* gene was used as an internal control. The reverse transcription was carried out using the cDNA kit (iNtRON Power cDNA Synthesis Kit) according to the manufacturer's instruction. CDNA was synthesized with 1 µg of RNA at 94°C for 5 min, followed by incubation at 40 °C for 60 min using Eppendorf PCR system. Real-time PCR was carried out using the prepared cDNA (100 ng) with each set of primers and SYBR® green master mix (Bioneer Company). Real-Time PCR conditions were 50 °C for 2 min, 95 °C for 10 min and 40 cycles, each having 95 °C for 15 s and 60 °C for 1 min in an The Corbett Rotor-Gene 6000 (QIAGEN, Germany).

Statistical analysis

Data was analyzed in MINITAB16. P Value and confidence intervals were <0.05 and 95%, respectively.

Results

MIC test found that 100 µg $mL^{-1}$of capsaicin as the highest concentration that did not affect the bacterial growth; however, zonula occludens toxin (*zot*) gene expression of the tested strain was significantly inhibited by capsaicin in a dose-dependent manner at sub-bactericidal concentrations (results from qRT-PCR assay) (Figure 1). The *rec*A gene did not show any significant difference in its expression with or without capsaicin.

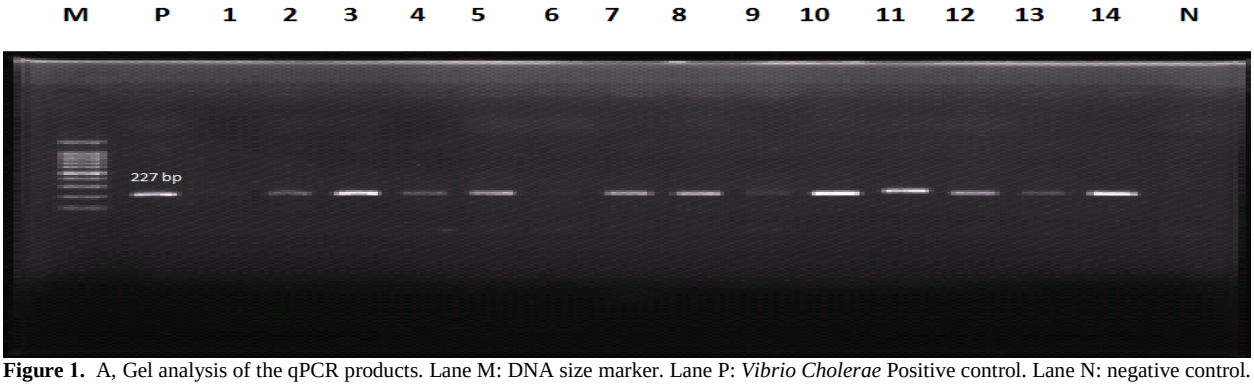

Figure 1. A, Gel analysis of the qPCR products. Lane M: DNA size marker. Lane P: *Vibrio Cholerae* Positive control. Lane N: negative control. Lane 1-14: *zot* positive isolate

Discussion

The cornerstone in the management of cholera is the use of oral rehydration therapy. The most important treatment is to replace the fluids and electrolytes that have been lost due to diarrhea (5). This is done either through oral fluid rehydration or intravenous fluid rehydration, in severe cases. In many cases, Antibiotic therapy has been demonstrated to reduce the duration of diarrhea, and they cannot be replaced simply by giving oral rehydration therapy. The increasing number of multidrug resistant pathogens and the accompanied rise in case fatality rates has

hindered the treatment of many infectious diseases including cholera (6). Capsaicin is a human carcinogen (7) and topical capsaicin is an established treatment option for various pain conditions (8). In 2010, Kalia *et al*. reported that capsaicin, a novel inhibitor of the NorA efflux pump, reduces the intracellular invasion of *Staphylococcus aureus* (9). Also in 2010, Chatterjee and et al. reported that *V.cholerae* growth can be inhibited by the use of 100 µg/ml of Capsaicin (10). In 2012, Santos *et al*. also reported that Streptococcus mutants growth can be inhibited by the use of 2.5 mg/ml of Capsaicin (11). Capsaicin is one of the active materials in red chili which can act as an antibacterial agent against bacterial pathogens such as *Helicobacter pylori and Bacillus* spp. Cichewicz *et al*. reported that Bacillus cereus, *Bacillus subtilis*, *Clostridium sporogenes*, *Clostridium tetani*, and *Streptococcus pyogenes* growth can be inhibited by capsaicin (12).

Capsaicin is one of the active compounds of red chilli that can drastically suppress *zot* gene expression and shows promising inhibitory activity against *Vibrio cholerae zot* production. Thus, routine intake of red chilli, which is easily available and inexpensive, may be an alternative approach to prevent and control symptoms of cholera.

Conflict of Interest

The authors declare that they have no conflict of interest in this work.

References

1. Higgins DA, Pomianek ME, Kraml CM, Taylor RK, Semmelhack MF, Bassler BL. The major Vibrio cholerae autoinducer and its role in virulence factor production. Nature. 2007; 450(7171): 883-6. PMID: 18004304

2. Butler SM, Camilli A. Going against the grain: chemotaxis and infection in Vibrio cholerae. Nat Rev Microbiol. 2005; 3(8): 611-20. PMID: 16012515

3. Muanprasat C, Chatsudthipong V. Cholera: pathophysiology and emerging therapeutic targets. Future Med Chem. 2013; 5(7): 781-98. PMID: 23651092

4. Rahman MA, Islam MS. Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole Leucas aspera extract. Asian Pac J Trop Biomed. 2013; 3(4): 273-9. PMID: 23620850

5. Boschi-Pinto C, Lanata CF, Mendoza W, Habte D. Diarrheal Diseases. In: Jamison DT, Feachem RG, Makgoba MW, Bos ER, Baingana FK, Hofman KJ, et al., editors. Disease and Mortality in Sub-Saharan Africa. 2nd ed. Washington (DC):2006.

6. Kutar BM, Rajpara N, Upadhyay H, Ramamurthy T, Bhardwaj AK. Clinical isolates of Vibrio cholerae O1 El Tor Ogawa of 2009 from Kolkata, India: preponderance of SXT element and presence of Haitian ctxB variant. PLoS One. 2013; 8(2): e56477. PMID: 23431378

7. Archer VE, Jones DW. Capsaicin pepper, cancer and ethnicity. Med Hypotheses. 2002; 59(4): 450-7. PMID: 12208187

8. Chrubasik S, Weiser T, Beime B. Effectiveness and safety of topical capsaicin cream in the treatment of chronic soft tissue pain. Phytother Res. 2010; 24(12): 1877-85. PMID: 21104944

9. Kalia NP, Mahajan P, Mehra R, Nargotra A, Sharma JP, Koul S, et al. Capsaicin, a novel inhibitor of the NorA efflux pump, reduces the intracellular invasion of Staphylococcus aureus. J Antimicrob Chemother. 2012; 67(10): 2401-8. PMID: 22807321

10. Saei-Dehkordi SS, Tajik H, Moradi M, Khalighi-Sigaroodi F. Chemical composition of essential oils in Zataria multiflora Boiss. from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem Toxicol. 2010; 48: 1562-7.

11. Santos MM, Vieira-da-Motta O, Vieira IJ, Braz-Filho R, Gonçalves PS, Maria EJ, et al. Antibacterial activity of Capsicum annuum extract and synthetic capsaicinoid derivatives against Streptococcus mutans. J Nat Med. 2012; 66(2):354-6. PMID: 21858615

12. Cichewicz RH,Thorpe PA. The antimicrobial properties of chile peppers (Capsicum species) and their uses in Mayan medicine. Ethnopharmacol. 1996; 52(2):61-70. PMID: 8735449