



ISSN NO. 2320-5407

Journal homepage: <http://www.journalijar.com>

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of CD –cholesterol on activity of α -hemolysin production by Methicillin resistant *Staphylococcus aureus* (MRSA)

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Manuscript Info

Manuscript History:

Received: 18 June 2014
Final Accepted: 29 July 2014
Published Online: August 2014

Key words:

Staphylococcus aureus , CD-cholesterol , α -toxin , inhibition , α -hemolysin.

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Abstract

Methicillin – Resistant *Staphylococcus aureus* (MRSA) represent a major problem in human medicine by causing both healthcare – associated and community associated infections .*S.aureus* secretes a number of host injurious toxins, among the most prominent of which is the pore –forming toxin (α -hemolysin) initially named based on its properties as a red blood cell lytic toxin . The present study focuses on the CD-cholesterol as a chemical inhibitor of α -toxin action .Some of bacterial infections are universally treated with antibiotics , which can eliminate the organism but cannot reverse the damage caused by bacterial products already presents .*S.aureus* produce proteins that directly or indirectly cause damage to the cell tissue that can result in reduced vision despite antibiotic treatment .CD-cholesterol is a good inhibitor of α -toxin activity in vitro and we considered a good antivirulence agent might be useful in prophylaxis and as adjuvants in antibiotic therapy for MRSA infection.

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Introduction

Staphylococcus aureus is a gram – positive human pathogen that is able to cause a multitude of diseases ranging in severity (Lowy , 1998) and it is the most common cause of bacterial keratitis and an important cause of other ocular infections (Sowak *et al.*, 2001). specific strains of bacteria found in the flora around the eye provide the source of organisms that infect the eye. (Speaker *et al.*, 1991). *Staphylococcus aureus* α –hemolysin (α –toxin , HLa) is the prototype for the class of small β - barrel pore –forming cytotoxins (PFTs)(Parker and Feil, 2005). α -hemolysin (α -HL) is a self –assembling ,channel –forming toxin that is produced as a soluble monomer by *S.aureus* strains α -hemolysin is one of the major toxins endowed with hemolytic ,cytotoxic , dermonecrotic and lethal properties(Qiu *et al.* ,2013). The alpha -toxin's mechanism of action indicates that the toxin enters the cytoplasmic membrane and moves laterally until seven subunits unite into a circular arrangement forming a pore in the cytoplasmic membrane(Menestrina *et al.* ,2001). β cyclodextrin compound prevents α –hemolysin –induced lysis of human alveolar epithelial cells . This protective effect does not result from the ability of the β - cyclodextrin, to impair formation of the oligomeric alpha –hemolysin on the cell surface ,supporting a role for this molecule in blockade of the lytic pore(Ragle *et al.*, 2010). Inhibitors of the alpha –toxin –mediated lysis of erythrocytes have been developed by inserting lipid molecules into a cyclodextrin ring (Weeks *et al.* ,2012). One such inhibition containing cholesterol has been shown to reduce the virulence of infections of the rabbit cornea (McCormick *et al.*, 2009) .CD application in the design of various novel delivery systems like liposomes, microspheres , microcapsules , and nanoparticles. In addition to their well –known effects on drug solubility and dissolution ,bioavailability ,safety , and stability (Challa *et al.* ,2005). α -hemolysin is a critical virulence factor that determines the severity of *S.aureus* infections when measured in mouse models (Szente and Szejtli, 1999) .

Material and Methods

-Bacteria

Methicillin resistant *Staphylococcus aureus* (MRSA) strain isolate from skin infection obtained from AL-Kindy Hospital –Baghdad- Iraq, and identified by conventional biochemical reactions and Gram staining, according to the criteria established by (Forbes *et al.*, 2002). Antimicrobial susceptibility of the isolate was tested by the disc diffusion method for Methicillin (10µg) (bioanalyse, Turkey) according to the clinical and laboratory Standards Institute (CLSI) guidelines (CLSI, 2009).

This isolate grown at 37°C in Tryptic Soy broth for 24 hrs, centrifugation and supernatant was taken and considered as crude toxin.

-Rabbit Blood Hemolytic assay

Rabbit blood hemolytic assay is a functional assay measuring the release of the hemoglobin from erythrocytes due to the hemolytic activity of HLa (Khodaverdian *et al.*, 2013).

Freshly collected rabbit erythrocytes were centrifuged to pellet and resuspended in normal saline with 0.02% gelatin; this washing procedure was repeated three times, washed erythrocytes were diluted to concentration of 10⁷ cells per ml. crude α -toxin was serially diluted twofold in microtiter plates, rabbit erythrocytes (10⁷ per well) were added to the crude α -toxin dilutions and the plates were incubated at 37°C until the erythrocytes in the control lacking α -toxin had settled the highest dilution producing red cell lysis was considered the end point (McCormick *et al.*, 2009).

-inhibition of hemolysis with CD- cholesterol

For inhibition of hemolysis supernatants of overnight cultures were serially diluted twofold in microtiter plates. erythrocytes (10⁷ per well) were mixed at 4°C with CD- cholesterol (0.1%) and then added to dilutions of the culture supernatant in microtiter plate. the dilutions producing lysis were observed after 30 minutes. all hemolysis inhibition assays were performed in duplicate (McCormick *et al.*, 2009).

Result and Discussion

The test CD- cholesterol could inhibit the action of α -toxin, aliquots of erythrocytes were mixed with CD-cholesterol 0.1% and then added to serial dilution of crude α -toxin. The crude α -toxin titer for the sample in CD-cholesterol was approximately (16). In contrast the hemolysis titer of the same amount of crude α -toxin incubated with erythrocytes (only) was continued of hemolysis until before last well (95) in microtiteration plate was stopped.

The present study demonstrates that CD-cholesterol is an effective inhibitor of α -toxin that can block the lytic action on erythrocytes. The mechanism by which CD-cholesterol inhibits α -toxin has not yet been demonstrated. The effectiveness of cholesterol as an inhibitor of the toxin is not unexpected, because Raff *et al.* (1978) previously demonstrated that α -toxin activity was weakly inhibited by a high concentration of hydrocortisone or methylprednisolone. It has been determined that caveolin, as found in lipid rafts, is an important glucocorticosteroid receptor that could bind molecules like methylprednisolone, hydrocortisone, or, in the present study, CD-cholesterol (Matthews *et al.*, 2008). Pany *et al.* (2004) and Vijayvargia *et al.* (2004) have demonstrated that α -toxin activity is dependent on caveolin-1. Vijayvargia *et al.* (2004) have also demonstrated that α -toxin activity on cells can be delayed by sequestering cholesterol, or inhibited altogether when cholesterol is depleted from cells. Therefore, a possible mechanism for the effectiveness of CD-cholesterol as an inhibitor of α -toxin could be that CD-cholesterol competes for the same cellular target as α -toxin. This competitive binding to the cellular target molecule would reduce the number of caveolin molecules available to α -toxin resulting in fewer cells being lysed as a consequence of α -toxin pore formation. There have also been reports of α -toxin inhibition by modified CD (Karginov *et al.*, 2007). Karginov *et al.* (2007) showed that hepta-6-substituted CD molecules can create a molecule that interferes with the lytic action of the toxin. These modified CD molecules are thought to have affinity for the α -toxin heptamer. Specifically, the modified CD molecules are thought to occlude the central pore of the toxin. Relative to the present study, one could speculate that the cholesterol molecule in CD-cholesterol interacts with the central portion of the α -toxin pore. Thus, the cholesterol in complex with CD is envisioned to occlude the toxin pore in a manner similar to modified-CD inhibitors of α -toxin described by Karginov *et al.* (2007).

Antivirulence agents inhibit the production of disease causing virulence factors but are neither bacteriostatic nor bactericidal. Antivirulence agents might be useful in prophylaxis and as adjuvants in antibiotic therapy for MRSA infections (Khodaverdian *et al.*, 2013).

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