The Effect Of Sublethal Concentrations Of Silver Nitrate On Alginate Production By Pseudomonas Aeruginosa

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Summary:

Background *: Many studies showed that the silver nitrate reduce the adherence of P. aeruginosa to the silver coated medical devices but non of these studies investigated the mechanism that inhibited the adherence of P.aeruginosa on silver coated medical devices .*
Objective: *Investigate the effect of sublethal concentrations of silver nitrate on alginate production and viscosity of slime in Pseudomonas aeruginosa.*
Methods *:Fifteen isolates were used in this study and one isolate was selected , which has more ability to produce large amount of alginate. The viscosity of the slime was expressed as slime index. The alginate was extracted by ethanol extraction method .*
Results: *The result showed that the amount of alginate decreased when the concentrations of silver nitrate were increased, and silver nitrate treated strain expressed more slime than untreated strain, but when compared to the different treatment together (0.125, 0.25 and 0.5x of the MIC), the obtained slime index decreased also when the concentrations of silver nitrate increased.*
Conclusion: *Prevents of medical devices related infections which coated by silver nitrate by P.aeruginosa due to efficient reduction of alginate and the viscosity of this alginate, which is consider one of the most important mechanism of bacterial adhesion of/*, aeruginosa.*
Keywords *: Pseudomonas aeruginosa , silver nitrate , sublethal concentrations , alginate , slime .*

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Introduction:

Bacterial adhesion represents a primary process of the pathogenic mechanism (l).Many bacterial ligands have been recognized as being important in this adhesive process . One of these, the alginic acid or alginate, an extracellular polysaccharide which considered as on of the important virulence factor produced by *P. aeruginosa* , the most common etiologic agent of sever infections in impaired and immunocompromised patients (2,3). This material has been shown to be as a antiphagocytic (4),inhibit bacterial binding to macrophage (5), stimulate antibody responses in patients (6), enhance adherence of the organism to mucosal surfaces(7),and on medical devices(8).

Many studies searched in preventing adherence of *P. aeruginoas* on medical devices by using antibacterial agents (9,10).Others studied the viability of the clinical use of medical devices coated with silver nitrate (AgNO3), which is showed good results in preventing medical devices related infections (ll,12).Moreover, these medical devices become widely used in many countries(13). But non of these studies investigated the mechanism that inhibited the adherence of *P.aeruginosa* on silver coated medical devices , so, the aim of this study is to test the effect of silver nitrate on the amount of alginate produces by mucoid strain of *P. aeruginoas* and on the viscosity of this alginate.

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Materials and Methods:

Bacterial isolation:

Fifteen isolates were used in this study ,three of them from burn, four from bacteremia, four from respiratory tract infection, three from ear infection and one from urine. All these isolates were grown on tryptic soy agar to select one isolate which has more ability to produce alginate (13).

Determination of minimal inhibitory concentrations (MIC):

The MIC of silver nitrate was determined by difold dilution method in agar using tryptic soy agar as recommended by Miles R.S. and Amyes S.G. (14).

The MIC was defined as the lowest concentration resulting in the complete inhibition of visible growth .

Viscosity measurement:

The viscosity of the slime was expressed as slime index , Bacterial growth was harvested from 0.5,0.25 and 0.125of the MIC plates in addition to the control plate after adding 10ml of phosphate buffer saline (PBS ,pH 7.2) to each plate . Then the viscosity was determined by

measuring the length of time the culture took to drain from a 10ml volumetric pipette divided by the length of time the distilled water took to drain from the same pipette (15).

Extraction of alginate:

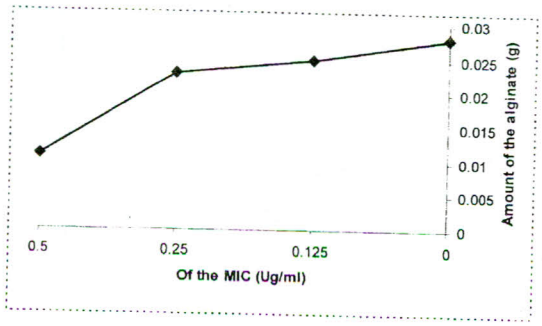
The alginate was extracted by using adapted method according to (16,17,18) and as following:

Mucoid isolate was grown on tryptic soy agar containing 0.5, 0.25 and 0.125 of the MIC of silver nitrate. The mucoid layer was collected with 10 ml of PBS (pH7.2) . then the cells were harvested by centrifugation (60000 Xg for 20 min at 4°C) .The clear supernatant was heated for 30 min at 80C to kill viable bacteria and to denature proteins. Disassociated and denature material was then removed from supernatant by centrifugation (12000 Xg for 20 min at 4°C) .The alginate in supernatant was extracted with 60ml of 95% ethanol. The precipitate was collected by centrifugation the sample at (10000 Xg for 10 min at 4°C) . The pellet was washed with 5ml of 100% ethanol ,then the ethanol was dried under a stream of air and the dry weight of the dried sample was determined.

Results:

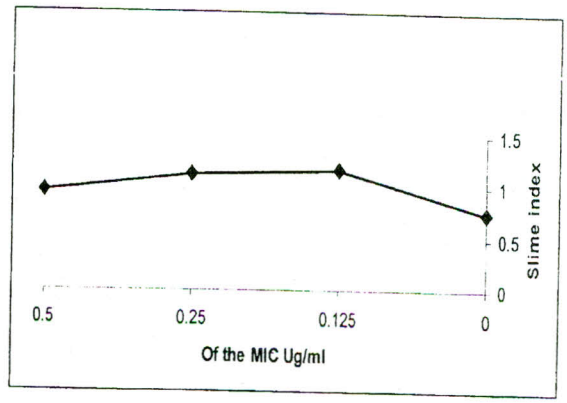
The preliminary results , to screen the mucosity of strains , revealed that the isolates which were collected from respiratory tract infections were more mucoid than other isolates. One of these isolates was chosen to test the effect of silver nitrate on alginate expression.

Generally , mucoid strains of *P. aeruginosa* constitutes the majority of the isolate from patients with chronic pulmonary infections caused by this bacteria (19). The non mucoid strains were isolated early in the infection, but during the course of the infection the predominant phenotype changed from nonmucoid to mucoid (20). The treatment with sublethal concentrations of silver nitrate (0.5, 0.25 and 0.125 of the MIC) efficiently decrease the amount of alginate gradually when the concentrations were increased , compared with the alginate that expressed under normal condition (Figure 1).



Figure(1): The effect of silver nitrate on alginate expression by *P. aeruginosa.*

When the effect of silver nitrate on viscosity of the slime we determined, the established result revealed that the slime index which obtained after treatment of strain was highest than the slime index of untreated strain, (Figure 2) . Indicating that the viscosity of the slime in treated strain more than the viscosity in the untreated strain, on other hand, when compared between different treatments together, the result shows that the viscosity decreased when the concentration increased.



Figure(2):Slime index of treated strain of ***P.aeruginosa*** with silver nitrate.

Discussion

The adherence of bacteria on solid surface , is the important first step in pathogenesis of medical devices related infections(21).There are many studies revealed that the *P. aeruginosa* has more ability than other pathogenic bactereia to adhere on solid surface (22,23). So ,the reduction in amount of alginate may explain the prevention of adherence of *P. aeruginosa* on these devices (23). Moreover, mucoid strains of *P. earuginosa* produce a polymannuronic acid depolymerase which is capable to degrade the expolysaccaride. Many studies state that , the sublethal concentrations of some materials like gentamicin and EDTA promoted the release of this polymerase by exponentially growing mucoid strain of *P. aeruginosa* (24), Therefore, the treatment with silver nitrate may promote the release of this depolymerase.

The results of viscosity may be explained according to the nature of alginate composition, Although alginate is chemically simple consisting of only mannronic acid and guluronic acid and it has been identified as o- acetylated polyuronic acid (25), but the degree of acetylation of bacterial alginate and the ratio of mannuronic acid to guluronic acid vary considerably and may be responsible for different physical properties as ion binding and gel formation (26). Moreover, several studies have been shown that the expolysaccaride produced by some strain of *P. aeruginosa* composed not only of classical alginate but also contain small

amount of natural sugar and amino sugar (27) , Therefore, increase amount of slime that was noted after the treatment with silver nitrate may be due to the influence of silver nitrate on the ratio of mannuronic acid to guluronic acid or to the presence or absence of other sugars. Furthermore, Treatment with the low concentration of silver nitrate (0.125x of the MIC) may trigger or activate the genes encoded enzymes of the alginate production, specially these genes normally silent or expressed the alginate in smallest amount (28) , but treatment with concentrations (0.25 and 0.5x of the MIC) , the metabolic pathways of alginate synthesis may block or depress the expression of the genes mediate the enzymes for alginate production , specially these genes responded to several environmental condition (28).

' Finally, The ability of P. *aeruginosa* to attach to surface and establish a biofilm can have far reaching consequences. The colonization of medical devices like indwelling catheter in hospitalized patient can lead to serious nosocomial infections. So, treatment with silver nitrate reduces the amount of alginate and viscosity then prevents the adherence of *P. aeruginosa* on clinically used medical devices.

References:

1- *Beachey, E.H.(1981). Bacterial adherence: Adhersion-receptor interactions mediating the attachment of bacteria to mucosal surface J.infect.Dis. 143:425-345.*

2- Hoiby, n.;doring,G. and Schiotz, P.O.(1987). Pathogenic mechanisms of chronic P. aeruginosa infections in cystic fibrosis patient. Antibio. Chemother. 39:60-76.

3- May, T.B.;Shinabarger,D.;Mahary,R.;kato,J.;Chu,L.;DeVault,J.D.; Roychoudaury,S.;Zielinsly,N.A.e?.a/. (1991). Alginate synthesis by P. aeruginosa : A key pathogenic factor in chronic pulmonary infection of cystic fibrosis patient. Clin. Microbiol. Rev. 4:191-206.

4- Laharraqe, P.F.; Corberand, J.X.; Fillola, G.: Gleiizes, B.J.; Fontanilles, A.M.; and Gyrarol, E .(1984).In vitro effect of the slime of P. aeruginosa on the function of human polymorphonuclear neutrophils. Infect. Immun. 44:760-762.

5- Oliver, A M. and Weir, D.M. (1983). Inhibition of bacterial binding to mouse macrophages by Pseudomonas alginate. J. Clin. Immun. 10:221-224.

6- Speert, T.W.; Thirkill, H.; Tarpay, M.; Flux, M and Rennert. O.M. (1984). Antibody to P. aeruginosa mucoid expolysaccarid and sodium alginate in cystic fibrosis sera. Pediatr. Res. 18: 431-433.

7- Ramphal, R. and Pier, G. B. (1985). Role of/$^{\delta}$, aeruoginosa mucoid expolysaccharide in adherence to tracheal cells. Infect. Imun. 46: 1-4.

8- Marrie, T.D. and Costerton, J. W. (1983). Electron microscopic study of urine dropping and urine collecting system. Arch. Intern. Med. 143: 1135-1141.

9- Trancassini, M.; Brenciaglia, M.L.; Ghezzi, M.C. ; Cipriani, P. and Filadora, F. (1992). Modification of P. aeruginosa virulence factors by subinhibiting concentrations of antibiotics. J. Chemother. 4: 78-81.

10- majtan, V. and Hybenova, D. (1996). Inhibition of P. aeruginosa alginate expression by inhibitory concentrations of antibiotics , Fiola Microbial. Praha. 41: 61-64.

11- Liedberg, H and Lundeberge, T. (1990). Silver alley coated catheters reduced catheter associated bacteria. Br.J.Urol. 65: 379-381.

12- Ahearn, D G. ; Grace, D. T. ; Jenninge, M.J.; borazjani, R.N.; Boles, K.J. ; Roswe, L. J.; simmons, R. B. and Ahanoty, E.N. (2000). Effect of hydrogel silver coating on in vitro adhesion to catheter bacteria associated with urinary tract infections. Curr. Microbiol. 41: 120-125.

13- Martinez-Maryinez,L.;Pascual,A and Parea, E.J. (1991). Kinetics of adherence of mucoid and non mucoid P. aeruginosa to plastic catheters. J.Med. Microbiol. 35:7-12.

14- Miles, R. S. and Amyes, S. G. (1996). Laboratory Control of Antimicrobial Theraoy : In Mackie and MaCareney Practical Medical Microbiology , by Collee,J.G. ; Fraser, A. G. ; Marmion, B. P. and Simmons, A. (14ed) Vol. I. Churchill Livingstone, New York, PP 151- 179.

15- Palumbo, A.S.(1972). Role of iron and silver in pigment and slime formation of P. aeruginosa J. Bacteriol. 111: 430-436.

16- Pedersen, S. S. ; Esperse, F. ; Hoiby, N. and Shand, G, H. (1989). Purification , characterization and immunological cross- reactivity of alginate produce by muciod P. aeruginosa from patient with cystic fibrosis. J. Clin. microbial. 27: 691-699.

17- Doig, Smith, N. R.; Todd, T. and Irvin, R. T. (1987) . Characterization of the binding of P. aeruginosa alginate to human epithelial cells. Infect. Immun. 55: 1517-1522.

18- Geers, T. A. and Baker, N. R. (1987). The effect of sublethal concentrations of aminoglycoside on adherence of P. aeruginosa to hamster tracheal epithelium. J. Antimicrobiol.Chemother. 19: 561- 568.

19- Rivera, M. and Nicotra, M. B.(1982). Pseudomonas aeruginosa mucoid strain , its significance in adult chest disease . Am. Rev. Respir. Dis. 126: 833- 836.

20- Pier, G. B. (1983). Pulmonary disease associated with P. aeruginosa in cystic fibrosis : current status of the host — bacterium interaction. J. Infect. Dis. 151:575-580.

22 21- Jansen, B. and Peter, G. (1991). Modern strategies in the preventation of polymer associated infections. J. Hosp. Infect. 19: 83-88.

22 - Lopez-Lopez,G.; Pascual, A. and Perea, E. J.(1991). Effect of plastic catheter material on bacterial adherence and viability. J. Med. Microbiol. 34: 349-353.

23-Gatter,N. ; Kohnen, W. and Janson, B. (1998). In vitro efficiency of hydrophilic central venous catheter loaded with silver to prevent microbial colonization , Zbl. Bakt. 287: 157-169.

24- Dunne, W. M. and Buckmire, F. L. (1985). Partial purification and characterization of a polymannuronic acid depolymerase produced by a cystic fibrosis patient. Applied Environ. Microbiol. 50: 562- 567.

25- Evans, L. R. and Linker, A. (1973). Production and characterization of the slime polysaccaride of P. aeruginosa . J. Bacteriol. 116: 915-924.

26- Sengha,S.S.; Anderson,A.J.; Hacking,a.J.and Dawes,E.A.(1989). The production of alginate by P.aeruginosa in batch and continuous culture . J. Gen. Microbiol. 135:795-804.

27- Mcarthur, H.A. and Ceri, H. (1983). Interaction of rat lung lactin with the expolysaccharides of P. aeruginosa . 42:574-578.

28- Schlictman, D.; Kubo,M.; Shanker,S. and Chakrabarty,A.M.(1995). Regulating nucleoside diphosphare kinase and secretable virulence factors in P.aeruginosa :Role of algR2 and algtt. 177:2469-2474.