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ORIGINAL ARTICLE

The effects of four different drugs administered through catheters on slime production in coagulase negative *Staphylococci*

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ABSTRACT

Objectives: Higher rate of slime production has been found in pathogen bacteria strains. Accordingly, the factors that contribute to higher slime production rate increase the infection risk, while the factors that reduce the slime production rate will reduce the infection risk. The effect of some drugs that are administered through catheters in intensive care units on slime production with coagulase negative Staphylococci was investigated.

Materials and methods: In this study, the effect of four different preparations containing Glyceryl trinitrate (Perlinganit®), Dexmedetomidine (Precedex®), Esmolol (Brevibloc®), and Propofol (Propofol®) on slime production of 24 *Staphylococcus epidermidis* strains isolated from blood cultures of patients, and reference strain were investigated. Slime production was determined using 'the quantitative microdilution plaque test' described by Christensen.

Results: Under controlled medium, eight strains formed slimes, and in the media containing esmolol, glyceryl trinitrate, dexmedetomidine, and propofol slimes were positive for five, 21, 15, and 18 strains, respectively. The rate of slime production in glyceryl trinitrate, dexmedetomidine, and propofol containing media were higher than that of the controls.

Conclusions: In the light of the results of this study, it is concluded that the drugs and/or additives increase the rate of slime production. The effects of the preparations administered through catheters on slime production should be investigated, and these effects should be kept in mind during their use. *J Microbiol Infect Dis 2012; 2(4): 150-154*

Key words: Slime Production, Coagulase Negative Staphyloccoci, Parenteral drugs

Kateter aracılığıyla verilen dört farklı ilacın koagülaz negatif Stafilokoklarda slime oluşuma etkisi

ÖZET

Amaç: Patojen bakteri suşlarında slime oluşturma oranı daha yüksek bulunmuştur. Slime oluşumunu artıran faktörler enfeksiyon riskini artırırken azaltanlar ise bu riski düşürmektedir. Çalışmamızda Yoğun Bakım Ünitelerinde kateter aracılığı infüzyon şeklinde uygulanan bazı ilaçların koagülaz negatif Stafilokok'larda slime oluşumuna etkisi araştırıldı.

Gereç ve yöntem: Glyceryl trinitrate (Perlinganit®), Dexmedetomidine (Precedex®), Esmolol (Brevibloc®) ve Propofol (Propofol®)'un slime oluşumuna etkileri 24'ü kan kültürlerinden izole edil ve biri American Type Culture Collection (ATCC) 12228 kodlu referans *Staphylococcus epidermidis* suşu üzerinde araştırıldı. Slime oluşumu Christensen tarafından tarif edilmiş olan "Kantitatif mikrodilüsyon plak testi" yöntemi ile belirlendi.

Bulgular: Kontrol kuyucuklarında 8 bakteri slime oluşturdu. Esmolol içeren besi yerinde 5, Glyceryl trinitrate'lı besiyerinde 21, dexmedetomidine olan besiyerinde 15, propofol'lü besiyerinde 18 bakteri slime pozitif bulundu. Glyceryl trinitrate, dexmedetomidine ve propofolün slime pozitifliğini kontrole göre anlamlı ölçüde arttırdığı saptandı.

Sonuç: Bu çalışmanın sonuçlarının ışığında; ilaç ve/ veya katkı maddelerinin slime üretimine etkili olabileceği sonucuna varıldı. Kateter yoluyla uygulanan preparatların etkileri araştırılmalı ve kullanımları sırasında bu etkilerinin olabileceği akılda tutulmalıdır.

Anahtar kelimeler: Slime üretimi; koagülaz negatif Stafilokok, parenteral ilaç

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INTRODUCTION

With the development of diagnostic and treatment tools, significant improvement has been attained in health care; however, complications associated with the use of these tools have arisen. Intravascular catheters with their wide range of use constitute the primary tools of such concern.

Intravascular catheters are the most common predisposing factor for bacteremia among nosocomial infections.[1] These infections are common in intensive care units (ICUs).[2] Catheter related bacteraemia, with an incidence rate of 20-30%, is the third most common nosocomial bacteraemia among others. Catheter related infections lead to a 10-20% increase in mortality and a prolonged hospitalization additional seven days.[3]

The pathogenesis of catheter infections is multifactorial and complex. Current data revealed that catheter infections develop -at first- by colonization of the microorganisms originating from the skin on the catheter tip.[1] In vitro studies with electron microscope have shown coagulase negative *Staphylococci* (CoNS) colonization on the irregular areas of the catheter in the first 30 minutes of catheter insertion. In one hour, micro colonies are formed. They continue to colonize in a single layer in the next 6-12 hours and then in multiple layers. These colonies are covered with a glycocalyx sheet and form the slime structure. This structure acts as a barrier against antibiotics and phagocytic cells.[2]

A higher rate of slime production has been determined in pathogen bacteria strains.[4] Numerous studies have investigated the slime presence with CoNS. In these studies, the rate of slime production has been reported to vary between 30% and 83%. For CoNS isolated from patients with bacteraemia, this rate was found to be as high as 93%.[4-8] Accordingly, it can be assumed that the factors that induce slime production increase the infection risk, whereas the factors that inhibit the slime production reduce the infection risk.

Slime factor is an extra cellular structure containing protein and carbohydrate and is responsible for adherence of microorganisms to host cells and artificial surfaces.[9] Bacteria are considered to be capable of using carbohydrates and particularly glutamine that are present in the culture medium to form this structure.[4] There is evidence on the stimulation of slime production with *S. epidermidis* strains by carbohydrates such as glucose, fructose, lactose, maltose, and sucrose, which are present in the medium.[4] Contrarily, various drugs such as oxybutynin, daptomycin, and acetylsalicyclic acid have been shown to inhibit slime production.[10-12]

Glyceryl trinitrate (GT) (Perlinganit®) is a potent vasodilator that decreases preload and afterload. It also activates coronary arteries. Esmolol (Brevibloc®) is a cardioselective beta1 receptor blocker with rapid onset. It is used to control rapid heartbeats or abnormal heart rhythms. This medicine is also used to treat fast heartbeat and high blood pressure during surgery, after surgery, or during other medical procedures. Dexmedetomidine (Precedex®) is a potent alpha2-adrenoceptor agonist. It decreases sympathetic tone and attenuates the stress responses to anesthesia and surgery; and also causes sedation and analgesia. Propofol (Propofol®) is a short-acting, intravenously administered hypnotic agent. It is an anesthetic typically used to produce general anesthesia or sedation. This study aimed to investigate the effects of four drugs which are infused via catheters in ICUs on slime production capability of CoNS:

MATERIALS AND METHODS

This study was performed on the Kırıkkale Universty Medical Faculty Microbiology Research Laboratory. Slime production effects of four different preparations containing glyceryl trinitrate (GT) (Perlinganit®), dexmedetomidine (Precedex®), esmolol (Brevibloc®), and propofol (Propofol®) in 24 strains of *Staphylococcus epidermidis* isolated from blood cultures of ICU patients and one reference strain (*Staphylococcus epidermidis*, American Type Culture Collection (ATCC) code: 12228) were investigated. Slime production was determined by using 'the quantitative microdilution plaque test' described by Christensen and coworkers.[5] The drugs used in the study were purchased from the drugstore.

Tryptic soy broth (TSB) (Oxoid; England) medium was used to dilute the bacteria. While the pure TSB was used as the control medium, the media containing TSB plus various drugs were used as the experimental mediums. Experimental mediums were containing the following end-perfusion concentrations of the agents used: 0.2 mg/ml glyceryl trinitrate; 4 µg/ml dexmedetomi-

dine; 2 mg/ml esmolol, and 2 mg/ml propofol in TSB. These concentrations were calculated from the dosage of these drugs were routinely used.

The preparations were transferred onto microplates and incubated at 37°C for 24 hours. At the end of the incubation period, the wells were emptied and washed four times with phosphate tampon solution. The plates were stained with crystal violet dye and read at 570 nm wavelength. The threshold values were calculated and slime producing bacterial strains was determined.[13]

The bacteria evaluated in the study were tested for their sensitivity to oxacillin according to Clinical and Laboratory Standards Institute (CLSI) standards by disk diffusion method. In addition, sensitivity to oxacillin was determined with agar screening test.

Statistical analysis was done by the help of a statistic program (SPSS for windows 13.5). Categorical variables were compared by chi-square test.

RESULTS

In the control medium, eight bacteria, including ATCC strain, produced slime. In the medium containing esmolol preparation, five bacteria; in the medium with GT preparation, 21 bacteria; in the medium with dexmedetomidine preparation, 15 bacteria, and in the medium with propofol, 18 bacteria were slime positive (Table 1).

Table 1. The slime positive/negative bacteria count in the pure (control) and drug containing tryptic soy broth media.

Media	Slime positive (%)	Slime negative (%)	Total (%)
TSB medium (control)	8 (32.0)	17 (68.0)	25 (100.0)
Glyceryl trinitrate (0.2 mg/ml) in TSB	21 (84.0)*	4 (16.0)	25 (100.0)
Dexmedetomidine (4 mg/ml) in TSB	15 (60.0)*	10 (40.0)	25 (100.0)
Esmolol (2 mg/ml) in TSB	5 (20.0)	20 (80.0)	25 (100.0)
Propofol (2 mg/ml) in TSB	18 (72)*	7 (28.0)	25 (100.0)

TSB: tryptic soy broth, *: Significantly higher than the control ($p<0.05$)

Glyceryl trinitrate, dexmedetomidine, and propofol were demonstrated to significantly increase slime positivity rate compared to the controls ($p<0.05$).

Four of the eight bacteria that were slime positive in the control media, were slime positive in all the other mediums. Two of the five bacteria that were determined to be resistant to oxacillin produced slime in all the mediums. No significant relationship was found between the sensitivity of the bacteria against the oxacillin, and slime positivity.

DISCUSSION

In recent years, the causative agents of nosocomial bacteraemia have changed. The incidence of four important pathogens CoNS, Candida species, Enterococcus species, and *Staphylococcus aureus* have increased. CoNS, particularly *S. epidermidis*, are the most commonly isolated bacterium in catheter-related infections and responsible for 28% of all nosocomial bacteremia.[1] In earlier studies, the increase in the incidence of this agent has been attributed to widespread use of prosthetic and permanent devices. For example, *S. epidermidis* bacteraemia was demonstrated in 90% of the patients with a history of intravascular catheter.[14] Despite various precautions aiming at reducing the incidence of intravascular catheter-related infections, it remains to be a serious risk in ICUs.

Adherence of microorganisms to the catheter depends on the physical characteristics of the catheter surface, adherence capability of the bacterium, host proteins, and intrinsic phenotypic characteristics of the bacterium that forms a biofilm layer. For instance, staphylococci adhere to polyvinyl, silicone, and polyethylene surfaces better than teflon and polyurethane surfaces. The adherence of CoNS to polymer surfaces is greater when compared with *S. aureus* and E. coli. Moreover, host proteins that cover the catheter in the vessel provide a strong adhering effect for bacteria.[15,16]

Production of slime by some strains of CoNS prevents them being captured and killed by polymorph nuclear leukocytes. In addition, it forms a matrix that antibiotics could bind before they contact bacterial cellular walls and thus, prevents the effects of antimicrobial agents on the microorganism.[1,2]

Various agents that reduce slime production have been defined. In a study evaluating the effects of heparin, streptokinase, vancomycin, minocycline, and EDTA on slime production, minocycline, EDTA, and minocycline EDTA mixture were found to reduce slime production by Methicillin-resistant *Staphylococcus epidermidis* significantly.[17] Gedik et al have reported that oxybutynin, which has a tertiary amine structure and chemically similar to protamine sulphate, used in incontinence treatment, reduces slime production of *S. epidermidis* in a dose dependent manner.[10] At routine treatment doses, daptomycin, a lipopeptide which has potent anti-gram positive activity, has been found to inhibit slime synthesis and destroy existing slime structure.[11] Similarly, Demirag et al have found that at five mM concentration, acetyl salycilate reduces slime production rate of *S. epidermidis* in dacron and polytetrafluoroethylene grafts.[12] On the contrary, some saccarides, primarily glucose, were shown to induce slime production.[4]

Christensen et al have stated that each bacterium acts differently in different media, and because in many media of routine use, no slime production is observed, this characteristic of bacteria may be overlooked. The authors have reported that TSB, which was also used in our study, is the best medium to detect slime producing potentials of the bacteria.[4] Accordingly, addition or exclusion of an agent into and from a medium and even the changes in the physical characteristics of the medium can cause differences in slime production potential of a bacterium. In this framework, although production of the basic substance of slime, polysaccharide, is genetically controlled, the definition of bacteria as slime positive or negative is largely dependent on the physical and chemical conditions of the experimental medium. Since slime production potential is parallel to the pathogenic capacity of bacteria, elucidation of each factor underlying this potential is of great importance. Elimination of the slime-inducing factor may prevent infection.

Of the four drugs evaluated in our study, three showed a significant potential for slime production compared to the control media. However, the drugs used were not in their pure forms but in their routinely used forms. Thus, it is difficult to clearly state whether the findings were affected by the active agents of the drugs or by various additives in their contents such as pH, and ionic load. Glucose in any medium has been shown to increase slime production. Accordingly, the effect of preparations with GT may have been due to 50.4 mg/ml glucose contained in the medium. Propofol has also been shown to increase slime production. The preparations with propofol contain various additives such as 5 mg/ml soy bean oil, 0.6 mg/ml egg lecithin, and 1.125 mg/ml glyceryl. The effects of these lipids remain to be investigated.

The drugs tested in this study are administered intravascularly through catheters in ICUs. To the best of our knowledge, no studies evaluating the slime producing effects of these drugs have been conducted to date. In the light of the results of our study, it can be stated that the drugs that significantly increase slime production (GT, dexmedetomidine, and propofol containing) may increase the risk of infection due to causative agents and/or additives. Nevertheless, controlled clinical trials are needed to confirm the results for practical applications. In addition, during production process of the preparations administered through catheters, the effects of these preparations on slime production should be investigated, and these characteristics of preparations should be considered in their routine use.

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