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IN VITRO SYNERGY BETWEEN GLYCOPEPTIDES AND CARBAPENEMS AGAINST METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Introduction: Glycopeptides have been used in the treatment of methicillin-resistant Staphylococcus aureus infections for the last 30 years. However, the emergence of staphylococcal isolates with reduced susceptibility to glycopeptides necessitates alternative therapies. A combination of well-known antibiotics may be an alternative approach to new drugs. The aim of this study was to investigate the synergy between glycopeptides and carbapenems, and glycopeptides and cefepime and whether there is any discrepancy between them.

Materials and Methods: A total of 30 MRSA isolates were studied. An E-test was performed to investigate the synergy of vancomycin-imipenem (VA-IPM), vancomycin-meropenem (VA-MEM), teicoplanin-imipenem (TEC-IPM), teicoplanin-meropenem (TEC-MEM), vancomycin-cefepime (VA-FEP), and teicoplanin-cefepime (TEC-FEP) combinations.

Results: Synergy was detected in 19 and 25 of the 30 strains for the VA-IPM and VA-MEM, and TEC-IPM and TEC-MEM combinations, respectively. VA-FEP synergy was detected in 19, and TEC-FEP synergy was detected in the 21 of the isolates.

Conclusion: Combination therapy may be an alternative to new drugs in the treatment of MRSA infections since in vitro studies provide promising results.

Key Words: Methicillin resistant Staphylococcus aureus, Synergy, Glycopeptides, Carbapenems.

METİSİLİNE-DİRENÇLİ STAPHYLOCOCCUS AUREUS SUŞLARINDA GLİKOPEPTİDLERİN VE KARBAPENEMLERİN İN VİTROSİNERJİK ETKİSİNİN ARAŞTIRILMASI

ÖZ

Giriş: Glikopeptidler, metisiline-dirençli Staphylococcus aureus suşlarının tedavisinde son 30 yıldır kullanılmaktadır. Glikopeptidlere duyarlılığı azalmış stafilokok suşların ortaya çıkmasıyla alternatif tedavilere gereksinim duyulmaya başlanmıştır. Bilinen antibiyotiklerin kombine kullanılması, yeni antibiyotiklere alternatif bir yaklaşım getirebilir. Bu çalışmanın amacı, glikopeptidlerle karbapenem kombinasyonunu ve glikopeptidlerle sefepim kombinasyonunun in vitro etkinliğini araştırmaktır.

Gereç ve Yöntem: Çalışmaya toplam 30 MRSA suşu alınmıştır. Vankomisin-imipenem (VA-İPM), vankomisinmeropenem (VA-MEM), teikoplanin-imipenem (TEC-İPM), teikoplanin meropenem (TEC-MEM), vankomisin-sefepim (VA-FEP), teikoplaninsefepim (TEC-FEP) kombinasyonlarının sinerjik etkinliğinin araştırılması amacıyla E-test stripleri kullanılmıştır.

Bulgular: VA-İPM, VA-MEM kombinasyonları için 30 suşun 19'unda, TEC-İPM ve TEC-MEM kombinasyonları için 30 suşun 25'inde in vitro sinerjik etki olduğu görülmüştür. VA-FEP kombinasyonu, 19 suş için, TEC-FEP kombinasyonu da 21 suş için sinerjik bulunmuştur.

Sonuç: Sorunlu infeksiyonların tedavisinde kombinasyon tedavisi yeni antibiyotiklere alternatif olabilir.

Anahtar Kelimeler: Metisiline dirençli Staphylococcus aureus, Sinerji, Glikopeptitler, Karbapenemler.

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INTRODUCTION

Infectious diseases caused by methicillin-resistant Staphylococcus aureus (MRSA) are currently a serious problem. Glycopeptides have been successfully used for the treatment of these infections for the past 30 years (1). Vancomycin is almost universally accepted as the drug of choice for the treatment of MRSA infections. However, vancomycin used alone kills staphylococci slowly, resulting in delayed recovery of patients with life-threatening infections (2-4). Glycopeptide intermediate and resistant S. aureus strains are emerging. The first report of a clinical S. aureus isolate that demonstrated reduced susceptibility to vancomycin, in 1997, has been followed by multiple reports of additional isolates seen in all parts of the world. In the United States, there have been 9 reported clinical cases of infection with S. aureus with intermediate resistance to vancomycin (VISA) as well as 2 known clinical cases of infection with S. aureus isolates fully resistant to vancomycin (VRSA) (5). Based on these findings, there is clearly a need for different antibiotic regimens. In this field, an alternative to the development of new classes of agents could be the use of combinations of well-known compounds (6). The synergy of glycopeptides with beta-lactams has been studied before (1, 6-9). A number of methods used to detect in vitro synergy between antibiotics have been described but checkerboard and time-kill curve methods are the most widely used (10). The epsilometer test (E test) is an agar diffusion method used for antimicrobial susceptibility testing. On the basis of in vitro studies, the method of synergy testing utilizing E-test strips appears to be a simple alternative to the other, labor-intensive synergy methods (9-12).

The aim of this study was to investigate the synergy between glycopeptides and carbapenems, and glycopeptides and cefepime. We also aimed to determine if imipenem or meropenem and vancomycin or teicoplanin has better synergistic effects than their counterparts in the same class.

MATERIALS AND METHODS

A total of 30 MRSA strains isolated from 30 patients attending Baskent University Hospital between 2002 and 2004 were studied. Nineteen isolates were obtained from blood, 6 from respiratory specimens, and 5 from surgical wound infections. Strains were identified to the species level by conventional methods (colony morphology, Gram stain characteristics, and coagulase reactions). All strains were methicillin resistant as determined by a disk diffusion method with a 1 µg oxacillin disk (13). The minimal inhibitory concentrations (MICs) of all strains were determined using E-test strips against vancomycin (VA), teicoplanin (TEC), imipenem (IPM), meropenem (MEM), and cefepime (FEP). Mueller-Hinton agar was used as the agar medium in all steps.

Table 1: FIC indices of beta-lactam and glycopeptide combinations.

	Mean FIC İndex	Synergy FIC≤0.5	Additive FIC=0.5-1	Indifference FIC=1-2	Antagonism FIC≥2
VA-IPM	0.32	19	0	11	0
VA-MEM	0.39	19	0	11	0
VA-FEP	0.41	19	0	11	0
TEC-IPM	0.31	25	0	5	0
TEC-MEM	0.32	25	0	5	0
TEC-FEP	0.39	21	0	9	0

MIC values of antibiotics were determined for each strain according to the CLSI (formerly NCCLS) criteria (13). The Mueller-Hinton agar plates were inoculated with the 0.5 McFarland inoculum and incubated with E-test strips of each antibiotic at 35 °C for 24 h. The MIC values for each antibiotic in the combination were recorded (MIC A, MIC B). None of the 30 strains had reduced susceptibility to glycopeptides. The IPM and MEM MICs were >32 mg/L, and FEP MICs were >256 mg/L for all strains. Staphylococcus aureus ATCC 29213 was used as the quality control strain in the MIC determination steps. The resultant MIC values of the control strain were within the ranges recommended in CLSI (13). Synergy testing by E test was performed as described elsewhere and also according to the manufacturer's recommendations (14,15). According to this method, the plates inoculated with bacteria were incubated for 1 h with the first E-test strip of the combination. After 1 h the strip was taken away from the plate and the E-test strip of the second antibiotic was put on exactly the same place (the concentrations of the two antibiotics should be in the same range) where the removed strip had been. The rationale for this technique is that the antibiotic in the strip is diffused through the agar in 30-60 h. Incubation of the strip after 1 h makes no difference in the results because the strip is used only for the reading scale then. The plates were incubated for 24 h at 35 °C and the MICs read were interpreted as the MIC of the two antibiotics together (MIC A+B).

Fractional inhibitory concentration (FIC) index = MIC A+B / MIC A + MIC A+B / MIC B

FIC index was interpreted as follows (10):

≤0.5 synergy
0.5-1 additive
1-2 indifference
≥ 2 antagonist.

Table 2: The distribution of the various types of combinations among MRSA strains.

Synergistic against All combinations	Number of strains
None of the combinations	15
Glycopeptide-carbapenem only	5
Glycopeptide-cefepime and Teicoplanin-carbapenem	4
Teicoplanin-carbapenem only	2
Total	30

RESULTS

As shown in Table 1, teicoplanin-beta lactam combinations seem to be more synergistic than vancomycin-beta lactam combinations. Another finding is that imipenem and meropenem "behaved" in the same way in the combinations, i.e. there was no discrepancy between VA-MEM and VA-IPM, and TEC-MEM and TEC-IPM combinations. Cefepime-glycopeptide combinations seem to be less effective than carbapenem-glycopeptide combinations. No additive or antagonistic interaction was determined (Table 1).

The distribution of the combination types for the strains is shown in Table 2. Fifteen of the 30 isolates were synergistic against all 6 combinations, whereas 5 of them were not synergistic against any of these. Four of them were synergistic against the glycopeptide-carbapenem combination but not against the glycopeptide-cefepime combination. Four of them were synergistic against glycopeptide-cefepime and teicoplanin-carbapenem combinations but not against the vancomycin-carbapenem combination. Two of them were synergistic against only the teicoplanin-carbapenem combination (Table 2).

DISCUSSION

MRSA strains represent a worldwide threat because of their virulence and their broad distribution in the hospital setting (6). The emergence of decreasing levels of glycopeptide susceptibility among these staphylococci has recently raised fears that effective antimicrobial treatment options of these isolates may soon be very limited (1). In this field an alternative to the development of new classes of agents could be the use of combinations of well-known compounds (6). In this study we analyzed the activities of 6 beta lactam-glycopeptide combinations against a set of 30 clinical isolates of MRSA by E test methodology.

The two most extensively used in vitro methods for detecting synergy, checkerboard and time-kill, have yielded mixed results in pertinent evaluations, obviously because the two methods measure different phenomena. The checkerboard technique, based upon MICs, reflects the inhibition of bacterial growth, whereas the time-kill methodology measures the extent of killing (10). The E test method for detecting synergy appeared to be a possible alternative to other in vitro methods not only because the agreement between results is good but also because it is simple to perform (10,11). There seems no perfect method to predict in vivo synergy. The growing litera-

ture on the detection of synergy by E test, especially for gram-negative bacteria (e.g., Pseudomonas aeruginosa), encouraged us to study the MRSA isolates by the same methodology (12,14-17). A limitation of this method is its inability to detect the exact MIC value if the MIC of the strain is greater than the maximum concentration on the strip. In our study the MIC values of all strains were >32 mg/L for carbapenems and >256 mg/L for cefepime. Based on the formula, this can only increase the FIC index, causing "pseudo" antagonism, but we observed no antagonistic effect. As all the strains are susceptible to glycopeptides, this is not a problem for glycopeptide MIC values. Synergistic effects of beta lactams with glycopeptides have been reported in studies investigating drugs for clinical use in treating MRSA. Our decision to study the glycopeptide-carbapenem and glycopeptide-cefepime combinations is based on the clinical practice that beta lactams and glycopeptides are usually combined in nosocomial infections because of multiple infections together (central venous catheter infection, urinary tract infection, pneumonia, etc.). Obviously, in vitro studies are of limited value in the prediction of in vivo synergy and further studies are warranted, but the preliminary in vitro data are promising. Totsuka and co-workers reported in vitro synergistic effects of vancomycin and imipenem for 34 of 36 (94.4%) MRSA strains isolated from clinical materials by the checkerboard method (8). Synergy with vancomycin and imipenem, and vancomycin and meropenem were determined in 24 and 23 of 27 isolates by checkerboard respectively and synergy for the same combinations were detected for 8 of 27 isolates by E test (17). This discrepancy was considered to depend on the fact that a precise FIC index cannot be determined in vancomycin-carbapenem combinations as the maximum concentration of imipenem or meropenem is 32 mg/L (17). Vancomycin-meropenem and vancomycin-imipenem were reported to be synergistic against 66% and 56% of MRSA strains, respectively, in a checkerboard study (6). Çokça and co-workers reported vancomycin-imipenem synergy for all MRSA strains studied (n=5) by the checkerboard method (9). Lozniewski and co-workers studied synergy by the checkerboard method and 3 of the 10 MRSA strains were found to be synergistic against a vancomycin-cefepime combination (7). Antagonism was not detected in the studies mentioned above, in parallel with our study. A synergistic effect was determined in 19 of the 30 isolates for vancomycin-imipenem, vancomycin-meropenem, and vancomycin-cefepime combinations, in 25 isolates for teicoplanin-imipenem and teicoplanin-meropenem combinations, and in 21 isolates for the teicoplanin-cefepime combination. Combinations with teicoplanin and carbapenem seem to be more synergistic than vancomycin-carbapenem combinations. We also aimed in this study to compare imipenem and meropenem, and vancomycin and teicoplanin, two comparator drugs of each class. Therefore, this appears to be an important outcome of this study. The combination with cefepime seems to be only slightly more synergistic (19 vs. 21). The teicoplanin-beta lactam combination was found to be more synergistic than the vancomycin-beta lactam combination against glycopeptide-intermediate S. aureus strains (18). No discrepancy was found between imipenem and meropenem in combinations. The number of strains in this study is too small to allow a general conclusion.

MRSA infections are increasing and becoming more difficult to treat day by day. Combination therapy may be an alternative to new drugs since in vitro studies provide promising results. Studying the synergy tests by simpler methods may guide clinicians in everyday practice. Further in vitro and in vivo studies are needed to clarify this issue.

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