İn Vitro Activity of Linezolid in Combination with Vancomycin, Teicoplanin, Fusidic Acid, and Ciprofloxacin Against Gram-Positive Pathogens

Gram-Pozitif Patojenler Üzerine Linezolidin, Vankomisin, Teikoplanin, Fusidik Asit ve Siprofloksasin ile Kombinasyonlarının In Vitro Aktivitesi

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Geliş Tarihi/*Received:* 04.09.2008
Kabul Tarihi/*Accepted:* 07.11.2008

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ABSTRACT Objective: In this study, it was aimed to investigate in vitro activities of linezolid combined with vancomycin, teicoplanin, fusidic acid, and ciprofloxacin against Gram-positive pathogens. **Material and Methods:** This study was conducted at Ege University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology. Clinical isolates used in this study were obtained retrospectively from the collection of Ege University Medical Faculty, Department of Microbiology and Clinical Microbiology, Bacteriology Laboratory. Minimal inhibitor concentrations (MICs) of test strains_methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), vancomycin intermediate *S. aureus* (VISA), Mu50 (vancomycin intermediate *S. aureus* strain), methicillin-susceptible *Staphylococcus epidermidis* (MSSE), methicillin-resistant *S. epidermidis* (MRSE), vancomycin-susceptible *Enterococcus faecalis* (VSE) and vancomycin-resistant *E. faecalis* (VRE)) were determined by microdilution method. Time-kill studies were performed over 24 h using an inoculum of 5 x 10^6 and 1 x 10^7 CFU/mL. Antibiotics were tested at concentrations 1x and 4 x MIC (minimum inhibitory concentration). **Results:** Synergy was detected between linezolid plus teicoplanin and fusidic acid at 1 x MIC concentrations in MSSA strain. In MRSE strain, linezolid showed the same effect with fusidic acid at 1xMIC. Antagonistic effect was found linezolid plus vancomycin and teicoplanin (4 x MIC) in VSE and linezolid plus ciprofloxacin (4 x MIC) in MRSE strain. **Conclusion:** In this study, linezolid plus fusidic acid appeared to be the most active combination against Gram-positive pathogens.

Key Words: Drug combinations; linezolid; drug synergism

ÖZET Amaç: Bu çalışmada, linezolidin vankomisin, teikoplanin, fusidik asit ve siprofloksasin ile kombinasyonlarının Gram-pozitif patojenler üzerine in vitro etkinliğinin araştırılması amaçlandı. **Gereç ve Yöntemler:** Bu çalışma, Ege Üniversitesi Eczacılık Fakültesi, Farmasötik Mikrobiyoloji Anabilim Dalı'nda gerçekleştirildi. Çalışmada kullanılan klinik izolatlar, Ege Üniversitesi Tıp Fakültesi, Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı, Bakteriyoloji Laboratuvarı'ndan retrospektif olarak sağlandı. Çalışma kökenlerinin (metisilin-duyarlı *Staphylococcus aureus* (MSSA), metisilin-dirençli *S. aureus* (MRSA), vankomisin orta düzeyde dirençli *S. aureus* (VISA), Mu50 (vankomisin orta düzeyde dirençli *S. aureus* kökeni), metisilin-duyarlı *Staphylococcus epidermidis* (MSSE), metisilin-dirençli *S. epidermidis* (MRSE), vankomisin-duyarlı *Enterococcus faecalis* (VSE) ve vankomisin-dirençli *E. faecalis* (VRE)) minimum inhibitör konsantrasyon (MİK) değerleri mikrodilüsyon yöntemi kullanılarak belirlendi. 24 saatlik zamana bağlı öldürme eğrisi yöntemi, 5 x 10^6 ve 1 x 10^7 CFU/mL bakteri inokulumu kullanılarak uygulandı. Antibiyotikler MİK ve 4 x MİK düzeylerinde test edildi. **Bulgular:** Metisilin-duyarlı *S. aureus* kökeninde linezolidin teikoplanin ve fusidik asit ile 1 x MİK (minimum inhibe edici konsantrasyon) konsantrasyonundaki kombinasyonunda sinerji saptandı. Metisilin-dirençli *S. aureus* kökeninde de linezolid, fusidik asit ile 1 x MİK konsantrasyonunda yine aynı etkileşimi gösterdi. Linezolid VSE kökeninde vankomisin ve teikoplanin ile, MRSE kökeninde ise siprofloksasin ile antagonistik etki gösterdi. **Sonuç:** Bu çalışmada, linezolidin Gram-pozitif patojenler üzerine en etkili kombinasyonu fusidik asit ile oluşturduğu görüldü.

Anahtar Kelimeler: İlaç kombinasyonları; linezolid; ilaç sinerjizmi

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Turkiye Klinikleri J Med Sci 2010;30(1):59-64

Nosocomial infections due to Gram-positive pathogens are associated with high morbidity and mortality. The main antibiotics used to treat these infections are the glycopeptides, but the emergence of vancomycin resistance in Enterococci and Staphylococci have recently been reported. Antimicrobial resistance among Gram-positive cocci has necessitated the rapid development of novel antimicrobial classes, such as the oxazolidinones.[1-5]

Linezolid is an antimicrobial agent of the oxazolidinones with potent activity against multidrug-resistant Gram-positive pathogens. It is a totally synthetic agent and acts through the inhibiton of protein synthesis and thus bacterial translation at the initial phase of protein synthesis. Existing mechanism of resistance to other ribosomal agents does not confer cross-resistance to linezolid. According to the results of time-kill assays, linezolid demonstrated bacteriostatic activity against staphylococci and enterococci.[1,5-8]

Antimicrobial combination therapy may be used to provide broad spectrum coverage, prevent the emergence of resistant mutants and obtain a synergy between both antimicrobial agents. Antimicrobial combinations are considered to be synergistic if the effect of combination is greater than the effect of either agent alone or greater than the sum of the effects of the individual agents. Antagonism may appear if the combination provides an effect less than the effect of either agent alone or less than the sum of the effects of the individual agents. There are few reports on in vitro activities of linezolid in combination with different antimicrobial agents.[6,9-11]

The objective of this study was to determine the in vitro activities of linezolid combined with other antimicrobial agents (vancomycin, teicoplanin, ciprofloxacin, fusidic acid) against drug-sensitive and drug–resistant *Staphylococcus aureus* and *Enterococcus faecalis*.

MATERIAL AND METHODS

This study was conducted at Ege University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology. Clinical strains of *S. aureus*, *S. epidermidis* and *E. faecalis* were obtained retrospectively from the collection of Ege University Medical Faculty, Department of Microbiology and Clinical Microbiology, Bacteriology Laboratory. The test organisms included one methicillin-resistant *S. aureus* (MRSA), one methicillin-susceptible *S. aureus* (MSSA), one vancomycin-intermediate *S. aureus* (VISA), Mu50 (vancomycin-intermediate *S. aureus* strain), one methicillin-resistant *S. epidermidis* (MRSE), one methicillin-susceptible *S. epidermidis* (MSSE), one vancomycin-resistant *E. faecalis* (VRE), and one vancomycin-susceptible *E. faecalis* (VSE).

Linezolid, vancomycin, teicoplanin, ciprofloxacin, and fusidic acid were provided by the manufacturers. MIC levels of linezolid were determined by microdilution method using Mueller-Hinton broth (MHB) (Merck) according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS).[12] *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as the reference strains.

For each strain, linezolid was studied alone and in combination with fusidic acid, ciprofloxacin, vancomycin, and teicoplanin at the MIC and 4xMIC (minimum inhibitory concentration) concentrations. According to Krogstad and Moellering method time-kill studies were performed in flasks containing MHB and single or combinations of antibiotics.[13] Overnight bacterial cultures were adjusted to a turbidity equivalent to that of a 0.5 McFarland standard, and further diluted to yield a starting inoculum ranging between 5×10^6 and 1×10^7 CFU/mL. In each case, an antibiotic-free control was prepared and the same procedure applied. At 0, 6, and 24 h of incubation at 37°C, samples were removed from test and growth-control cultures and appropriately diluted with cold 0.9 % of sodium chloride and inoculated onto Mueller-Hinton agar plates. After incubation at 37°C for 24 or 48 h, bacterial colonies were counted. All time-kill studies were performed twice.

Synergy or antagonism was defined as an increase or decrease of at least 100-fold compared to the effect of the single most active agent at 24 or 48 h.

RESULTS

Antibiotics' MICs for strains were shown in Table 1. All strains were susceptible to linezolid. Mu50, VISA and VRE strains showed increased MICs of vancomycin and teicoplanin. MRSA, Mu50, VISA, and VRE strains were resistant to ciprofloxacin. All strains were susceptible to fusidic acid, except for MRSE.

In MSSA strain, linezolid demonstrated additive effect with vancomycin and synergistic effect with teicoplanin and fusidic acid at MIC concentrations after 24 h of incubation (Figure 1). About MRSA and Mu50, linezolid and fusidic acid combination showed additive effect at MIC and 4xMIC at 24 h. In MRSE strain linezolid, in combination with vancomycin (1 x MIC) and teicoplanin (4 x MIC), showed an additive interaction. Although linezolid expressed synergistic effect with fusidic acid at MIC, it demonstrated antagonistic effect with ciprofloxacin at 4 x MIC after 6 and 24 h in MRSE strain (Figure 2). Additive effect was detected when teicoplanin added linezolid at 4 x MIC concentration in MSSE. Antagonistic interaction was observed linezolid plus vancomycin and teicoplanin (4 x MIC) in VSE during 24 h (Figure 3). In VRE strain, additive effect was exhibited between linezolid and fusidic acid at the MIC concentration.

DISCUSSION

Linezolid is a novel antibiotic with excellent activity against multidrug resistant Gram-positive organisms. Despite the reported low frequencies of mutation to linezolid in vitro, the development of resistance among both Enterococci and Staphylococci during linezolid therapy has been described previously. In clinical settings, linezolid may be combined with other antimicrobial agents in order to increase the bactericidal activity of therapy, prevent the emergence of drug-resistant subpopulations and provide a complementary antimicrobial spectrum.[6,9,14]

Recently published studies have investigated the in vitro activities of linezolid in combination with different antimicrobial agents. No synergistic combination including linezolid in combination with gentamicin, vancomycin, ciprofloxacin, rifampin, fusidic acid, or fosfomycin was observed in those in vitro studies. In time-kill curves, the combination appears to be antagonistic when linezolid was combined with gentamicin, vancomycin, ciprofloxacin, and fosfomycin. In this context, new investigations into the effects of drugs in combination with linezolid may be worthwhile.[9,10]

In this study, synergy was detected between linezolid plus teicoplanin and fusidic acid at 1xMIC concentrations in MSSA strain. In MRSE strain, linezolid showed the same effect with fusidic acid at 1 x MIC. Antagonistic effects were found between linezolid plus vancomycin and teicoplanin (4 x MIC) in VSE and linezolid plus ciprofloxacin (4 x MIC) in MRSE strains. Linezolid showed additive interaction with vancomycin, teicoplanin and fusidic acid in some strains.

TABLE 1: MIC values of antibiotics against the test strains.

	MIC (µg/mL)				
Strain	**Linezolid**	**Vancomycin**	**Teicoplanin**	**Ciprofloxacin**	**Fusidic acid**
MSSA	2	0.5	0.5	0.125	0.125
MRSA	2	0.5	0.5	8	0.06
Mu50	0.5	8	16	8	2
VISA	0.5	8	8	32	2
MSSE	1	0.5	0.5	0.125	0.06
MRSE	0.5	1	2	0.06	16
VSE	0.5	0.25	0.03	1	2
VRE	1	64	32	32	4

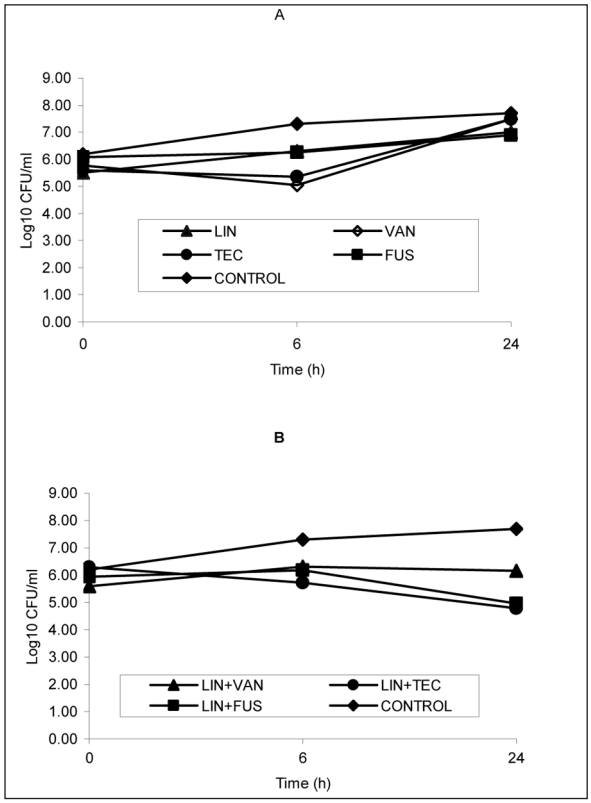

FIGURE 1: Time-kill curves of MSSA strain exposed to antimicrobial agents at the MIC concentration. A:▲ linezolid alone, ◊ vancomycin alone, ● teicoplanin alone, ■ fusidic acid alone, ◆ control. B: ▲ linezolid plus vancomycin (additive interaction), ● linezolid plus teicoplanin (synergistic interaction), ■ linezolid plus fusidic acid (synergistic interaction), ◆ control.

Sweeney and Zurenko have investigated the in vitro activities of linezolid combined with 35 antimicrobial agents against Staphylococci and Enterococci using the checkerboard method.[11] Although the combinations predominantly showed no difference against MRSA and MSSA strains, linezolid combined with amoxicillin was synergistic against three MRSA strains and linezolid plus imipenem showed synergism against one MSSA strain. In VRE and VSE strains, linezolid in combination with other antimicrobials predominantly showed no difference. While linezolid plus teicoplanin was synergistic against one VSE strain, an antagonistic activity was detected between linezolid plus ofloxacin against one VSE, and linezolid plus sparfloxacin against one VRE strain.

In a study conducted in France, in vitro combination of linezolid with vancomycin, ciprofloxacin, gentamicin, fusidic acid, and rifampin was investigated on five MSSA and five MRSA strains.[10] When combined with fusidic acid, gentamicin or rifampin, linezolid did not show any synergistic effect. When linezolid was combined with vancomycin and ciprofloxacin, a slight antagonism was observed.

Jones et al, have investigated the in vitro activities of oxazolidinones plus gentamicin and vancomycin.[4] While combined with gentamicin, oxazolidinones showed synergistic effect against streptococci but not against staphylococci.

Jacqueline et al, tested linezolid alone and in combination with gentamicin, vancomycin, and rifampicin against MRSA strains.[9] Linezolid had antagonistic effect with gentamicin and when combined with rifampicin, it showed an additive interaction.

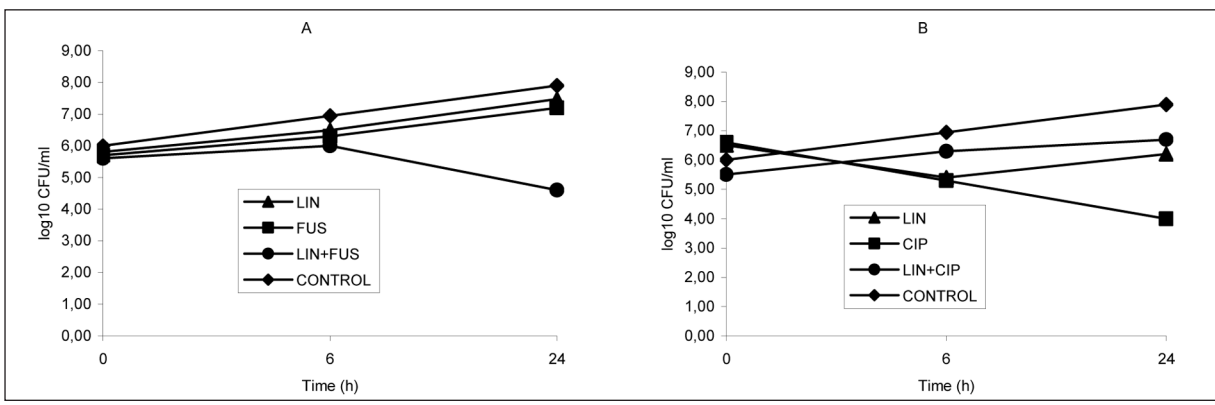

FIGURE 2: Time-kill curves of MRSE strain exposed to antimicrobial agents at 4xMIC concentration. A:▲ linezolid alone, ■ fusidic acid alone,● linezolid plus fusidic acid (synergistic interaction), ◆ control. B: ▲ linezolid alone, ■ ciprofloxacin alone, ● linezolid plus ciprofloxacin (antagonistic interaction), ◆ control.

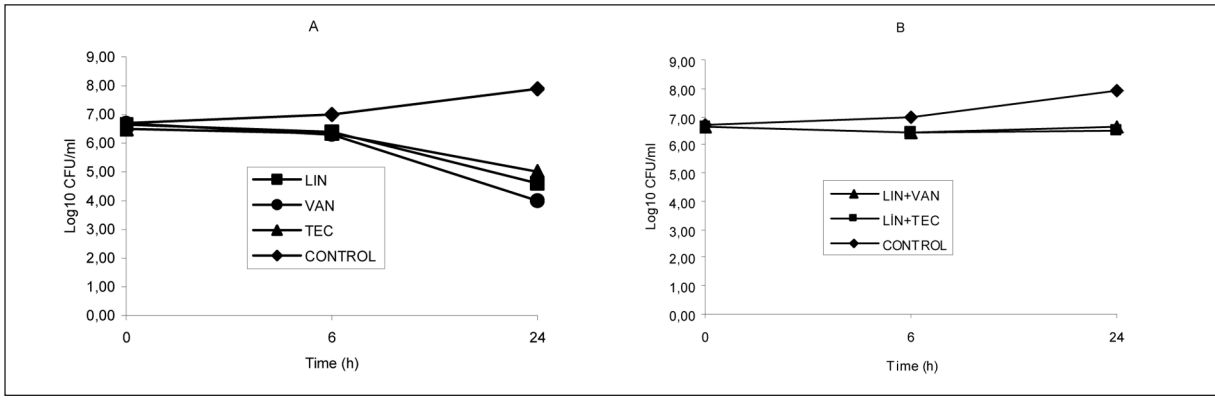

FIGURE 3: Time-kill curves of VSE strain exposed to antimicrobial agents at 4xMIC concentration. A: ■ linezolid alone, ● vancomycin alone, ▲ teicoplanin alone, ◆ control. B: ▲ linezolid plus vancomycin (antagonistic interaction), ■ linezolid plus teicoplanin (antagonistic interaction), ◆ control.

In another study conducted by the same investigators, synergistic effect was demonstrated between linezolid and imipenem.[6]

Mülazımoğlu et al have reported that linezolid showed no synergistic or antagonistic effect with rifampin and vancomycin.[15] In another study, linezolid reduced the bactericidal effect of fosfomycin against a MRSA strain.[16]

To date, as seen above, there have been few in vitro studies related to combinations of linezolid with other drugs. While Sweeney et al, found synergistic effect in VSE strain between teicoplanin and linezolid, we saw the same effect in MSSA strain.[11] In this study, antagonistic effect was observed with linezolid plus vancomycin and ciprofloxacin as seen in a study conducted in France.[10]

CONCLUSION

Antibiotic combinations that include linezolid can be used in the treatment of multiresistant Gram-positive infections. We have detected synergistic and antagonistic interactions between linezolid and some other drugs. Linezolid plus fusidic acid appeared to be the most active combination against Gram-positive pathogens. Consequently, in vivo studies are needed to validate the in vitro observations reported here with time-kill experiments.

Acknowledgement

We wish to thank Assist. Prof. Dr. Nevin Koyuncu from Ege University, Faculty of Letters, Department of English Language and Literature for her editing the article.

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