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Antagonism Between Antibiotics Frequently Used in The Treatment of *Staphylococcus aureus* Infections and The Hypertensive Drug L-Captopril

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Research Article

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ABSTRACT

Objective: Captopril is a long-acting human angiotensin-converting enzyme (ACE) inhibitor that has been used to treat hypertension and heart failure for many years. In this study, it was aimed to determine the antimicrobial activity of L-captopril on Methicillin-Resistant *Staphylococcus aureus* MRSA and Methicillin-Sensitive *Staphylococcus aureus* (MSSA) clinical isolates and, demonstrate the combination activity of captopril with ciprofloxacin (CXP) and gentamicin (GEN), which are among antistaphylococcal chemical agents.
Method: The minimum inhibitor concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the microdilution technique in 96-well microtiter plates. The activity of L-captopril and CXP or GEN combination against MRSA and MSSA clinical isolates was determined by the micro-broth checkerboard assay method.
Results: The captopril MIC value was determined to be 2.5 mg/ml in all bacteria strains tested. The captopril/CXP combination had an indifferent effect in the other strains tested except for one MRSA isolate. The captopril/GEN combination had an antagonistic effect in all strains studied and increased the MIC 2-4 fold. The captopril/GEN combination was found to reduce the bactericidal activity of gentamicin.
Conclusions: The study results suggest that exposure to these drugs may lead to multidrug resistance in *S. aureus* bacteria. Especially in the hypertensive patient group, the induced resistance in any *S. aureus* infections should be taken into consideration while using captopril and, it should be considered that this antagonism may cause an increase in *S. aureus* infections.

Keywords: Captopril, checkerboard, ciprofloxacin, gentamicin, *Staphylococcus aureus*

Staphylococcus aureus Enfeksiyonlarının Tedavisinde Sıklıkla Kullanılan Antibiyotikler İle Hipertansif İlaç L-Captopril Arasındaki Antagonizma

ÖZ

Amaç: Captopril yıllarca hipertansiyon ve kalp yetmezliğini tedavi etmek için kullanılan uzun etkili insan anjiyotensin dönüştürücü enzim (ACE) inhibitörüdür. Bu çalışmada L-captopril'in, Metisiline Dirençli *Staphylococcus aureus* (MRSA) ve Metisiline Duyarlı *Staphylococcus aureus* (MSSA) klinik bakteri izolatlarına karşı antimikrobiyal aktivitesinin belirlenmesi ve captoprilin antistafilokok kimyasal ajanlar arasında yer alan ciprofloxacin (CXP) ve gentamisin (GEN) ile kombinasyon aktivitesinin gösterilmesi amaçlanmıştır.
Yöntem: Minimum inhibitör konsantrasyon (MİK) ve minimum bakterisidal konsantrasyon (MBK) değerleri 96 oyuklu mikrotitre plakalarında mikrodilüsyon tekniği ile belirlenmiştir. L-captopril'in CXP ve GEN kombinasyonlarının MRSA ve MSSA klinik izolatlarına karşı aktivitesi, mikrodilüsyon dama tahtası yöntemi ile tespit edilmiştir.
Bulgular: Çalışılan tüm bakteri suşlarında captoprilin MİK değeri 2.5 mg/ml olarak belirlenmiştir. Bir MRSA izolatı dışında çalışılan diğer suşlarda captopril/CXP kombinasyonu önemsiz etki göstermiştir. Çalışılan tüm suşlarda captopril/GEN kombinasyonu antagonistik bir etki göstermiş ve MİK değerinde 2-4 kat oranında artışa neden olmuştur. Captopril/GEN kombinasyonunun gentamisinin bakterisidal aktivitesini azalttığı tespit edilmiştir.
Sonuç: Çalışma sonuçları bu ilaçların kullanımının *S. aureus* bakterilerinde çoklu ilaç direncine yol açabileceğini düşündürmektedir. Özellikle hipertansif hasta grubunda captopril kullanılırken herhangi bir *S. aureus* enfeksiyonunda indüklenen direnç dikkate alınmalı ve bu antagonizmanın *S. aureus* enfeksiyonlarında artışa neden olabileceği göz önünde bulundurulmalıdır.

Anahtar sözcükler: Captopril, dama tahtası, ciprofloxacin, gentamisin, *Staphylococcus aureus*

Süreç

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Introduction

Staphylococcus aureus is an opportunistic pathogenic bacteria that cause numerous infectious infections such as pneumonia, sepsis, and endocarditis [1]. Community-acquired and nosocomial infections caused by this bacteria are frequently encountered. Over time, these pathogenic bacteria have developed resistance to antibiotics such as aminoglycosides, fluoroquinolones, macrolides, and tetracyclines [2]. Although both community-acquired infections and hospital-acquired infections are common, the emergence of multidrug-resistant strains such as MRSA (Methicillin-Resistant *S. aureus*) makes treatment difficult [1]. Length of the treatment period and modality of *S. aureus* infections are planned according to the type of infection, and the presence/absence of resistant strains [3].

The β-lactam group antibiotics are the oldest antibiotic agents most commonly used in the clinic but increased antibiotic resistance has significantly reduced the effectiveness of these antibiotics. The most important factor in β-lactam resistance is serine amino acid and zinc-dependent Metallo-β-lactamases (SBLs/MBLs), which are commonly found in various gram-positive and gram-negative pathogenic bacteria and catalyze β-lactam hydrolysis [4]. Research in the literature indicates that in 95% of clinical *S. aureus* isolates, different types of β-lactamases are expressed, which is the primary cause of β-lactam antibiotic resistance [5]. The expression rate of MBLs in *S. aureus* increased to 20%-30% result of the spread of resistant plasmids among the strains. Therefore, the development of new MBL inhibitors is urgently necessary to combat the increasing resistance to-lactam antibiotics [6].

Captopril is a human angiotensin-converting enzyme (ACE) inhibitor that has been used successfully for many years in the treatment of hypertension and heart failure [7]. The structural and functional evidence has proven the antibacterial efficacy of captopril. Captopril causes the death of bacteria by inhibiting the MBLs (Metallo-β-lactamases) and N-succinyl-l,l diaminopimelic acid desuccinylase enzymes (DapE) of bacteria [8,9].

The studies on the antibacterial properties of captopril are limited in the literature. This study was aimed to determine the antimicrobial activity of L-captopril on MRSA and MSSA (Meticillin Susceptible *Staphylococcus aureus*) clinical isolates and to reveal the CXP-GEN combination activity among antistaphylococcal agents.

Material and Methods

Chemical Agents and Microbial Strain

Five MRSA clinical isolates (ciprofloxacin and gentamicin resistant bacteria) and eight MSSA clinical isolates (ciprofloxacin and gentamicin sensitive bacteria) were used in this study, which were isolated from various clinical samples at Sivas Cumhuriyet University Practice and Research Hospital, Clinical Microbiology Laboratory, in Sivas, Turkey. L-captopril, Ciprofloxacin (CXP), and Gentamicin (GEN) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA).

The strains were identified by MALDI TOF-MS (Bruker Biotyper Daltonik, Germany) system from colonies that were planted on sheep blood agar media (Becton Dickinson, USA) and grew in an overnight incubation at 35±2°C. *In vitro* susceptibilities of the strains to antibiotics were determined with BD-Phoenix automated system (Becton Dickinson, USA).

L- Captopril, Ciprofloxacin and Gentamicin Susceptibility Testing

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined using the microdilution method in 96-well microtiter plates (MBC) [10]. Firstly, L-captopril (100 mg/ml), CXP (2.56 mg/ml), and GEN (2.56 mg/ml) were dissolved in deionized water, and the stock solutions were prepared. Two-fold serial dilutions of captopril and antibiotics were prepared using Cation-Adjusted Mueller – Hinton Broth (CAMHB). The antibacterial activity of CXP, GEN, and L-captopril were assessed over concentration ranges of 0.125–128 µg/ml and 0.004–5 mg/ml respectively. Microplates with the 12th-row wells were employed for growth and sterility control. The bacterial isolate concentrations were adjusted to McFarland 0.5 concentration using sterile saline and overnight culture. It was diluted to a final bacterium concentration of 5×10^5 CFU/mL ($1x10^8$ cells/ml) in the wells. The prepared microplates were incubated at 37ºC for 18-24 hours and the first well without growth was determined as the MIC value [11].

To detect the MBC value, 10 µl of samples were obtained from wells where MIC values were examined but no growth was seen and inoculated onto Mueller Hinton Agar (MHA) medium. The MBC value was determined as the minimum number indicating that after 24 hours of incubation at 37°C, no growth was seen and that the original inoculum was destroyed at a rate of 99.9% [12].

Synergistic Activity

The Clinical & Laboratory Standards Institute (CLSI) (2018) devised the micro broth checkerboard method to test the *in vitro* antimicrobial efficiency. For this reason, we used this method to test the combined effect of L-captopril, CXP, and GEN against MRSA and MSSA bacteria. The assays were carried out following Pillai et al. protocol's [13]. Briefly, the combined effects of captopril and antibiotics for each isolate were tested in a 96-well microplate.

The ranges of captopril and antibiotics concentrations tested were 0.3125–5 mg/ml and 0.125–128 µg/ml respectively. Working concentrations of solutions in CAMHB have been prepared. The final bacteria concentration in the wells was added to the wells at $5x10^5$ cells/ml. The microplates were incubated for 24 hours at 37ºC, and the results were assessed after that time.

The single and combined MIC values of both drugs were used to determine the fractional inhibitory concentration index (FICi).

The following formula was used to compute FICi values for two antimicrobial drugs that were used together:

$$\sum \text{FICi: FICA+FICB} = \frac{(MIC-CC)}{(MIC-CA)} + \frac{(MIC\ of\ CXP/GC)}{(MIC\ of\ CXP/GA)}$$

MIC-CC : MIC of L-Captopril in combination
MIC-CA : MIC of L-Captopril alone
GC : GEN in combination
GA : GEN alone

The FICi was interpreted in the following way: Synergistic effect: FICi ≤ 0.5, additive effect: 0.5 <FICi ≤ 1, indifferent effect: 1<FICi<2, antagonistic effect: FICi≥2 [14].

Isobolograms were plotting using GraphPad Prism, ver. 8.0 software (GraphPad Software, Inc. California, CA) to present the FICindex of the combinations [15].

Statistical Analysis

The research was statistically analyzed using the IBM-SPSS 25.0 (IBM Co., Armonk, NY, ABD) program. At least three duplicates of each experiment were conducted. The level of statistical significance was set at p<0.05.

Results

Antibacterial Potency of Ciprofloxacin, Gentamicin, and Captopril

Captopril MIC value was determined to be 2.5 mg/ml in all strains tested. CXP, and GEN, MIC values for MRSA and MSSA clinical isolates are given in table 1.

Combinational Antibacterial Effect

In our study, the effectiveness of Captopril/CXP and Captopril/GEN combinations was investigated against MRSA and MSSA clinical isolates. The results of the checkerboard test are expressed in table 2.

In addition test results were also represented by the isobologram generated by plotting the FICs of Captopril and CXP/GEN in figure 1.

Discussion

In our study, it was determined that L-captopril had an antibacterial effect on all Multi-Drug Resistance (MDR) *S. aureus* isolates tested. In a limited number of studies, it has been reported that captopril has inhibitory activity against several classes of MBL enzymes, including zinc metalloproteases. It has also been found that captopril has antimicrobial activity against *Escherichia coli* bacteria [16,17].

Table 1. MIC and MBC values of MRSA and MSSA strains

Bacterial Strains	CXP (µg/ml)		GEN (µg/ml)		L-Captopril (mg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
MRSA-1	128	128 <	64	128	2.5	5
MRSA-2	64	128	32	64	2.5	5
MRSA-3	32	64	16	32	2.5	5
MRSA-4	128	128 <	128	128	2.5	5
MRSA-5	64	64	64	128	2.5	5
MSSA-1	0.125	0.25	0.5	1	2.5	5
MSSA-2	1	2	0.125	0.25	2.5	5
MSSA-3	0.125	0.25	0.5	1	2.5	2.5
MSSA-4	0.25	0.5	1	2	2.5	2.5
MSSA-5	0.125	0.25	0.25	0.5	2.5	5
MSSA-6	0.125	0.25	0.5	1	2.5	5
MSSA-7	0.125	0.25	0.25	0.5	2.5	5
MSSA-8	0.125	0.25	0.5	1	2.5	2.5

Table 2. *In vitro* antibacterial and synergistic activity of L-Captopril and CXP/GEN combination against MRSA and MSSA strains

Bacterial Strains	CXP (µg/ml)				GEN (µg/ml)			
	$MICC_{cxp}$	$MICC_{captopril}$	ΣFIC	Activity	$MICC_{gen}$	$MICC_{captopril}$	ΣFIC	Activity
MRSA-1	128	5	3.0	ant	128	5	4	ant
MRSA-2	64	0.31	1.12	ind	128	5	6	ant
MRSA-3	32	0.625	1.25	ind	64	5	6	ant
MRSA-4	128	1.25	1.5	ind	128	5	3	ant
MRSA-5	64	0.31	1.12	ind	128	5	4	ant
MSSA-1	0.125	0.31	1.12	ind	2	5	4	ant
MSSA-2	1	0.625	1.25	ind	1	5	6	ant

ΣFIC; FICindex sum, MICC; Combination MIC, CXP; Ciprofloxacin, GEN; Gentamicin, Ant; antagonistic, Ind; indifferent. Synergistic effect: FICi ≤ 0.5, additive effect: 0.5 <FICi ≤ 1, indifferent effect: 1<FICi<2, antagonistic effect: FICi≥2 [14].

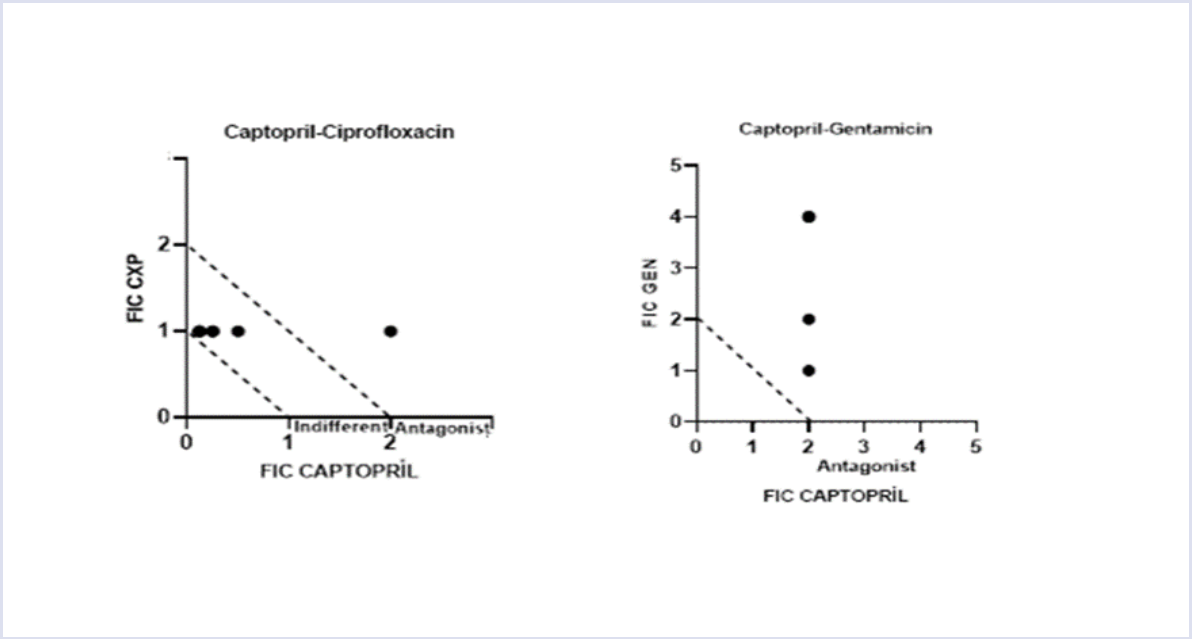

Figure 1. Isobologram of the FICIndex results of the combinations (indifferent (1 <FICindex <2) and antagonistic (FICi≥2) effects of Gen/CXP and Captopril against MRSA and MSSA clinical isolates

Gentamicin (GEN) is an aminoglycoside antibiotic with broad-spectrum antibacterial action that is still being used in preclinical and clinical trials [18]. GEN inhibits the function of bacterial ribosomes and blocks protein synthesis, leading to the death of bacteria [19]. However, the frequent use of antibiotics often leads to high levels of antibiotic resistance and reduces the therapeutic activity of GEN. Ciprofloxacin is a fluoroquinolone antibiotic that is frequently preferred in the treatment of MRSA infections. Shortly after entering the use of these bacteria, it has become rapidly resistant to ciprofloxacin as well [20]. The studies in literature have indicated that more than 89% of MRSA strains are resistant to ciprofloxacin [21]. In recent years, increased resistance against antibiotics has become a serious problem that limits treatment options. Developing new antibiotics is a time-consuming task that requires a lot of manpower and resources. Therefore, combination therapy is under consideration which provides efficient use of existing antibiotics and increases antibiotic activity [22]. Although these combination therapies often have synergistic activities, antagonistic interactions can be seen [23,24].

The Captopril/CXP combination showed an indifferent effect in the other MRSA strains tested, except for one MRSA isolate. Captopril/GEN combination showed an antagonistic effect in all strains tested. Our data proved that the gentamicin antibiotic used in combination with captopril increased the MIC values 2-4 times in all strains and, that it reduced the bactericidal activity of the gentamicin. In many studies investigating the combination of GEN with different antimicrobial agents against *S. aureus* bacteria; synergistic, additive, indifferent antagonistic effects were observed [25-30].

Captopril is a drug that shows antibacterial activity on many microorganism groups due to its MBL inhibitory properties. It has been determined that it shows synergistic properties as a result of its use with β-lactam antibiotic groups. Brem et al. [31] observed that D-Captopril, the stereoisomer of L-C captopril, increased the efficacy of meropenem against MBL-producing *E. coli, Klebsiella pneumoniae, Serratia marcescens,* and *Pseudomonas aeruginosa* bacterial strains.

Conclusion

The study results propounded that exposure to these drugs may lead to multidrug resistance in *S. aureus* bacteria. Therefore, resistance induced in any *S. aureus* infections should be taken into consideration while using captopril, especially in the hypertensive patient group and it should be considered that this antagonism may cause an increase in *S. aureus* infections. In particular, each case should be evaluated individually in antistaphylococcal prophylactic treatment. In addition, it is recommended that the use of these antibiotics, which are widely used in the treatment of *S. aureus* infections and have high antibacterial activity with captopril, should be investigated in more clinical terms.

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