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Antibacterial Activity of Allicin Alone and in Combination with β-Lactams against *Staphylococcus* spp. and *Pseudomonas aeruginosa*

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Abstract Allicin is one of the most effective compounds isolated from garlic showing antibacterial activity. Determination of MIC alone or in combination with cefazolin/oxacillin against *Staphylococcus* spp. or with cefoperazone against *Pseudomonas aeruginosa* showed that allicin alone did not have good antibacterial activity (MIC_{90} >512 μg/ml) but it facilitated antibacterial activity of all three β-lactams tested at subinhibitory concentrations. In the presence of 1/8 to 1/2 the MIC of allicin, the MIC_{90} values of cefazolin, oxicillin, and cefoperazone were reduced by 4～128, 32～64, and 8～16 fold, respectively. Thus, allicin-β-lactam combinations offer promise of clinical utility especially if synergism is demonstrated by *in vivo* experimental studies.

Keywords allicin, cefazolin, oxacillin, cefoperazone, synergy

Introduction

Staphylococcus aureus, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* are significant clinical pathogens [1～3]. With the increasing use of antibiotics in the clinic, multidrug resistant strains become frequently encountered [4～6], making treatment with a single agent more and more difficult to cure infections. Attempts have been made to cope with this problem by combination therapy [7].

Garlic is the bulb of *Allium sativum*, which is a Liliaceae Allium plant [8～11]. It is claimed to have antiseptic, anti-inflammatary, and antiflatulent properties. Allicin is the main effective antibacterial ingredient of garlic [12]. In the present study, *in vitro* antibacterial activity of allicin alone and in combination with cefazolin or oxacillin against Staphylococci spp. or with cefoperazone against *P. aeruginosa* was investigated. Synergistic effect was observed between allicin and each of the three β-lactams studied.

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Materials and Methods

Strains and Agents

Twenty each of *S. aureus* and *S. epidermidis* clinical isolates and 31 *P. aeruginosa* isolates were used in this study. All isolates were verified by VITEK. *S. aureus* ATCC29213 and *P. aeruginosa* ATCC27853 were used as quality controls. Allicin Injection (30 mg/2 ml) was purchased from Lukangchenxin pharmaceutical limited company (Shandong, China) and was diluted by M–H broth for each experiment. Cefazolin (purity 99.3%, Batch No. 0421-9603), oxacillin (purity 90.4%, Batch No. 0335-9403), and cefoperazone (purity 96.5%, Batch No. 130420-200304) standards were obtained from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). Cefazolin stock solution was prepared in phosphate buffer (pH 6). Oxacillin and cefoperazone were prepared in sterile distilled water.

Checkerboard Microdilution Assay

The initial concentration for each bacterial suspension was 1.5×10^5 cfu/ml. The final drug concentration ranged 1024～1.0 mg/liter for allicin, 32～0.031 mg/liter for

Table 1 Interaction of allicin combined with cefazolin/oxacillin against 20 each of clinical isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* by checkboard microdilution assays

Compound(s)	*S. aureus*				*S. epidermidis*			
	MIC_{50}[a]	FIC	MIC_{90}[a]	FIC	MIC_{50}[a]	FIC	MIC_{90}[a]	FIC
Allicin	512	NA[b]	1024	NA[b]	512	NA[b]	1024	NA[b]
Cefazolin	0.5	NA[b]	16	NA[b]	2	NA[b]	4	NA[b]
Oxacillin	0.25	NA[b]	8	NA[b]	1	NA[b]	16	NA[b]
Allicin+cefazolin[c]	128/0.125	0.5	256/0.125	0.26	64/0.25	0.25	256/1	0.5
Allicin+oxacillin[c]	64/0.0625	0.38	256/0.125	0.37	64/0.125	0.25	128/0.5	0.16

[a] MIC values were in mg/liter. [b] NA: not applicable. [c] MICs in combination are espressed as [allicin]/[antibiotics].

cefazolin and oxacillin, and 128～0.125 mg/liter for cefoperazone. Two-fold dilutions of each drug or drug combination were tested. Results were read after plates were incubated at 37°C for 24 hours. MIC_{90} and MIC_{50} were determined as the lowest concentration of the drugs (alone or in combination) that inhibited growth by 90 or 50% respectively of the strains tested [13]. The fractional inhibitory concentration (FIC) index is defined as the sum of the MIC of each drug when used in combination divided by the MIC of the drug used alone. Synergistic effect was recorded when FIC indexes≤0.5; partial synergy when FIC>0.5 but <1.0; additive when FIC=1.0; indifferent when FIC>1.0 but <4.0, and antagonistic when FIC≥4.0 [7].

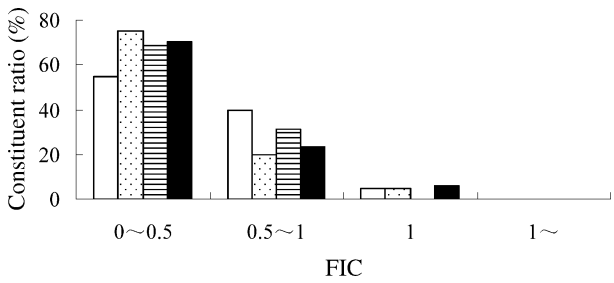

Fig. 1 FIC index distribution of allicin combined with cefazolin/oxacillin against 20 each of clinical isolates of *Staphylococcus aureus* or *Staphylococcus epidermidis*.

□ Allicin+cefazolin to SA, ⊡ allicin+oxacillin to SA, ⊟ allicin+cefazolin to SE, ■ allicin+oxacillin to SE.

Results

Enhancement of Antibacterial Activity of Cefazolin and Oxacillin against *S. aureus* and *S. epidermidis* by Allicin

The results of the checkboard analysis are summarized in Table 1. The MIC_{90}s of cefazolin and oxacillin alone were 16 and 8.0 mg/liter, respectively with *S. aureus*, and 4.0 and 16 mg/liter, respectively with *S. epidermidids*. When allicin was added at 1/8 or 1/4 MIC to the β-lactam regimens, the MIC_{90} of cefazolin decreased by 128- and 4-fold, respectively with *S. aureus* and *S. epidermidis* while that of oxacillin decreased by 64- and 32-fold with *S. aureus* and *S. epidermidis*, respectively. Synergism was observed for each β-lactam antibiotics/allicin combination since all FIC values for MIC_{90} were less than 0.5 (Table 1). FIC value distribution among 40 *Staphylococcus* spp. was presented in Fig. 1. Fifty-five to seventy-five percent of isolates had FIC values less than 0.5, 20～40% isolates gave FIC between 0.5 and 1 among which most had values just above 0.5, and no indifferent or antagonism effect was observed.

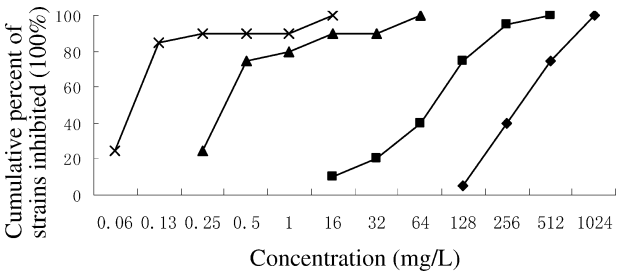

Fig. 2 Cumulative percent of strains inhibited of allicin and cefozolin against 20 clinical isolates of *Staphylococcus aureus*.

◆ Allicin, ▲ cefozolin, ■ allicin in combination, × cefozolin in combination.

The cumulative percent inhibition curve also showed improved activity of allicin and cefozolin combination when compared with either compound alone (Fig. 2). Similar results were obtained with allicin and oxacillin combinations (data not shown). These data indicate that synergism may be the main reason for improved activity of

Table 2 Interaction of allicin combined with cefoperazone against 31 clinical isolates of *Pseudomonas aeruginosa* by checkboard microdilution assays

Compounds	Susceptible isolates (n=17)				Resistant isolates (n=14)			
	MIC_{50}[a]	FIC	MIC_{90}[a]	FIC	MIC_{50}[a]	FIC	MIC_{90}[a]	FIC
Allicin	256	NA[b]	512	NA[b]	128	NA[b]	512	NA[b]
Cefoperazone	16	NA[b]	32	NA[b]	64	NA[b]	128	NA[b]
Allicin+cefoperazone[c]	64/0.5	0.28	256/2	0.56	64/4	0.56	128/16	0.38

[a] MIC values were in mg/liter. [b] NA: not applicable. [c] MICs in combination are espressed as [allicin]/[antibiotics].

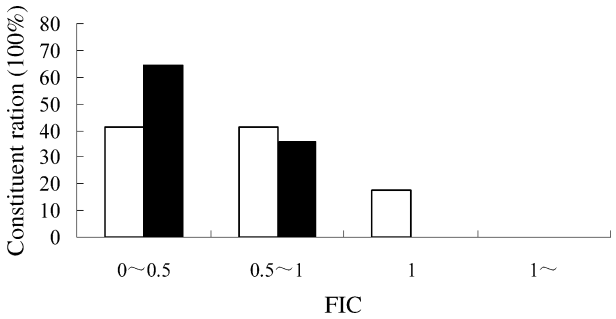

Fig. 3 FIC index distribution of allicin combined with cefoperazone against 17 sensitive susceptible and 14 drug-resistant *Pseudomonas aeruginosa* isolates.

□ Sensitive strains, ■ drug resistant strains.

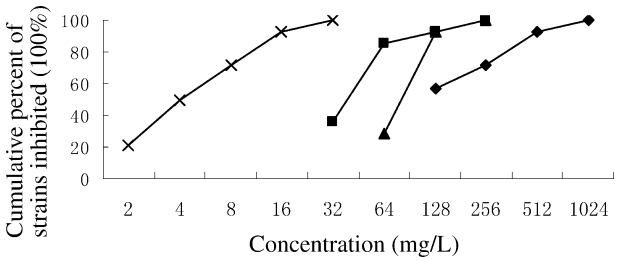

Fig. 4 Cumulative percent of strains inhibited of allicin and cefoperazone against 14 strains of drug resistant *Pseudomonas aeruginosa*.

◆ Allicin, ▲ cefoperazone, ■ allicin in combination, × cefoperazone in combination.

allicin and cefazolin or oxacillin combinations against Staphylococci.

Enhancement of Antibacterial Activity of Cefoperazone by Allicin against *P. aeruginosa*

The MICs of cefoperazone were 4-fold higher for drug resistant strains, which exceed the blood levels attainable for clinically recommended dosage. When combined with 1/4~1/2 MIC of allicin, the MICs of cefopeazone reduced by 8~32 fold over that of cefoperazone used alone (Table 2), making drug-resistant strains treatable with clinically recommended dosage. Synergy and partial synergy was evident with the allicin - cefoperazone combination because FIC values ranged between 0.28~0.56 (Table 2) and because 41~64% of isolates had FIC less than 0.5, 36~41% gave FIC values between 0.5 and 1, and no resistant and only less than 18% of susceptible isolates had FIC values of 1 (Fig. 3). No isolate gave a FIC value of more than 1 (Fig. 3). The cumulative percent inhibition curve also demonstrated that the combination regimen had much better activity than either compound alone with both resistant (Fig. 4) and susceptible isolates (not shown).

Discussion

Infections caused by Gram-positive cocci and *P. aeruginosa* are increasing both in hospitals and in general community [1~6, 14~18]. The efficacy of many antibiotics for treatment of severe infections has become quite limited due to the development of resistance. Allicin, one of the main effective antibacterial ingredients isolated from garlic, has also shown little adverse reaction, no antigenicity, and some immuno-modulation activity [19, 20]. Many published data indicate that allicin has certain *in vitro* antibacterial activity but the MICs are often relatively high, limiting its clinical utility [21~23]. Consistent with these previous reports, our data also show relatively poor activity against all three bacterial species tested when allicin is used alone. Little difference in allicin MIC was observed between susceptible and resistant isolates of *P. aeruginosa* but the MIC with *P. aeruginosa* was slightly lower than with Staphylococci. When allicin was combined with cefazolin, oxacillin, or cefoperazone, it stimulated the activities of these β-lactam antibiotics in a synergistic fashion. The combined MIC_{90} was brought down as many

as 128-fold for cefazolin - allicin combination with *S. aureas* compared with cefazolin alone at an allicin concentration of only 1/4 MIC. MICs with other allicin - β-lactam combinations against other bacteria tested were also reduced by 4-fold, making traditional dosages even effective against resistant strains of *P. aeruginosa*. These data encourage further studies with allicin plus other antimicrobial classes and *in vivo* animal experiments to validate this interesting finding before clinical test can move forward. Despite of lack of knowledge for the underlying mechanism of the synergistic effect of allicin - β-lactam combinations, the potential for use of such combinations clinically is huge since it may be able to make some untreatable resistant infections treatable at currently recommended dosages that are often marginally effective against resistant strains when used alone. We are now testing other combinations and preparing animal work for validation of the synergistic effect.

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