IN VITRO SYNERGISTIC ACTIVITY OF IMIPENEM AND FOSFOMYCIN AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

KOHKI TAKAHASHI and HARUSHIGE KANNO

Clinical Laboratory, Chiba University Hospital, Chiba, 1-8-1 Inohana, Chiba 280, Japan

ZUIMEI MIYAZAKI

Department of Chest Medicine, Institute of Pulmonary Cancer Research,
School of Medicine, Chiba University

The *in vitro* activity of imipenem in combination with fosfomycin against 17 clinical isolates of methicillin-resistant *Staphylococcus aureus* from hospitalized patients was investigated by checkerboard and time-kill studies. Of the 17 strains tested, checkerboard studies revealed synergy in 14 (82.3%) and indifference in 3 (17.6%) ; the time-kill studies also revealed synergy in 14 of 14 strains at both 24 and 48 h. The combination of imipenem and fosfomycin may be useful for methicillin-resistant *Staphylococcus aureus* infections.

Key words : MRSA, Imipenem (IPM), Fosfomycin (FOM), Effect of combination

INTRODUCTION

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming increasingly prevalent[1,2]. At present, vancomycin remains the primary drug used to treat these infections[3,4], but its use involves several drawbacks[5,6]. Imipenem is the first clinically useful member of the carbapenems, a new class of antimicrobial agents that are unique among β-lactams both for their chemical structure and their broad antibacterial spectrum. Since its development from thienamycin as the N-formimidoyl derivative, imipenem has been studied extensively in recent years, and the data available about its *in vitro* antibacterial activity have been reviewed repeatedly[7,8]. However, in these studies, investigations of its interaction with other antimicrobial agents are relatively limited. Overall, synergism has been demonstrated in combinations of imipenem with aminoglycosides against enterococci[9~12] and *Listeria monocytogenes*[10] and less frequently against staphylococci, enteric Gram-negative bacteria, and nonfermentative bacteria[13~17]. Conversely, imipenem has shown antagonistic interaction with other β-lactam antimicrobial agents against *Pseudomonas aeruginosa*[18~20] and *Serratia marcescens*[21] and with chloramphenicol against *Klebsiella pneumoniae*[22]. Fosfomycin is a broad-spectrum bactericidal antibiotic that acts against Gram-positive and -negative bacteria by inhibiting the first step in bacterial cell wall synthesis[23]. It has been shown to be active against *Staphylococcus aureus*[24,25] including penicillin- and methicillin-resistant strains. It has little or no reported toxicity on serum proteins and does not bind to them[23,26].

In this study, MRSA strains isolated from clinical material were tested for susceptibility to imipenem, and fosfomycin. Combinations of imipenem with fosfomycin were then determined both by checkerboard and by time-kill studies.

MATERIALS AND METHODS

MRSA used in this study were isolated from hospitalized patients at Chiba University Hospital. MRSA were decided according to NCCLS[27]. Namely we decided as MRSA *Staphylococcus*

aureus with MIC of ≥ 4 μg/ml against oxacillin.

Of the 30 strains of MRSA tested, only 17 strains with antibiotic MICs within the range of drug concentrations tested were used in this study. The antibiotics were kindly provided by the manufacturers as reagent-grade powders. Imipenem was obtained from Merck (Japan) Co., Ltd., Tokyo; and fosfomycin was obtained from Meiji Seika Co., Ltd., Tokyo, Japan. Methicillin and oxacillin were obtained from Banyu Co., Ltd., Tokyo, Japan. Potency of imipenem, fosfomycin, methicillin, and oxacillin is 956, 747, 850, and 837 μg/ml, respectively. Solutions of each antibiotic were made on the day when they were tested. Serial twofold dilution of methicillin, oxacillin, fosfomycin, and imipenem were made at 512 to 0.25, 128 to 0.06, 64 to 1, and 32 to 0.03 μg/ml in cation-supplemented Mueller-Hinton broth (CSMHB) (Difco Laboratories, Detroit, Mich.) with 2% NaCl supplementation, respectively, and dispensed into microdilution trays, which were then stored frozen at $-70°C$.

Broth microdilution testing was performed by methods published by the National Committee for Clinical Laboratory Standards[27]. The inoculum of the organism was adjusted from a log-phase culture to give a final concentration of 5×10^5 CFU/ml (5×10^4 CFU per well). The covered trays were incubated at 35°C for 24 h[27]. The MIC was defined as the lowest concentration of antibiotic that yielded no visible growth. The MIC_{50} and MIC_{90} were the lowest concentrations that inhibited 50 and 90% of the strains.

In vitro combinations of imipenem with fosfomycin were assayed by checkerboard studies in 17 strains. The studies were performed by standard microdilution technique in CSMHB with 2% NaCl supplementation. Broth microdilution trays were prepared with an automated system (MIC-2000, Dynatech Laboratories, Inc., Alexandria, Va.) which dispensed 0.1 ml samples of twofold dilutions of imipenem, and fosfomycin in CSMHB with 2% NaCl supplementation. The trays were stored frozen at $-70°C$ until ready for use. MRSA strains from a log-phase culture in CSMHB with 2% NaCl supplementation at 35°C were diluted to

10^7 CFU/ml (MacFarland 0.5) with sterile water. Each well of the checkerboard microdilution broth trays was inoculated with 0.005 ml of the suspension, using a 96-prong automated inoculator (Dynatech Laboratories). Thus, the final inoculum was approximately 5×10^6 CFU/ml (5×10^4 per well). Inoculated trays were covered and incubated for 24 h at 35°C. After 24 h of incubation, the microdilution trays were examined for growth with a mirror reader (Dynatech Laboratories). MICs were recorded as the lowest concentration of antibiotic which suppressed visible growth after 24 h of incubation.

We defined the combination effect by using the fractional inhibitory concentration (FIC) index at the combination point of the lowest concentration of antibiotics suppressing growth after 24 h of incubation at 35°C. We interpreted a combination effect to be synergistic if the FIC index was ≤ 0.5 and antagonistic if the FIC index was > 4[28]. Intermediate values were interpreted as indifference.

The time-kill curve method was used to study the combination of imipenem with fosfomycin. Fourteen strains and CSMHB with 2% NaCl supplementation were used. The antibiotic concentrations were as follows: imipenem, 15 μg/ml; fosfomycin, 32 μg/ml; and the combination of imipenem and fosfomycin, 15 and 32 μg/ml, respectively. These are nearly average concentrations obtainable in serum[29, 30]. The inoculum was approximately 10^6 CFU/ml, diluted from a log-phase culture. All tubes were incubated at 35°C. Samples from each tube were collected at 0, 3, 6, 24, and 48 h of incubation and quantitatively subcultured on blood agar plates. The timed quantitative subculture trays were then incubated at 35°C for 24 h and the viable number of MRSA was determined for each sampling time. Using the time-kill curve method and combined antibiotics, *in vitro* bactericidal synergy was defined as a minimum of a 100-fold decline in CFU/ml in comparison to the effect of the most active single agent at each sampling time, antagonism as a 100-fold increase in CFU/ml in comparison to the least active single agent, and

Table 1. MICs of 17 strains of methicillin-resistant *Staphylococcus aureus*

Strain no.	MIC (μg/ml)			
	methicillin	oxacillin	fosfomycin	imipenem
34	32	64	64	1
35	32	16	64	0.5
41	256	>128	64	2
43	32	16	32	8
44	256	>128	32	1
45	64	16	64	0.5
48	256	>128	64	2
49	32	16	64	4
53	128	>128	32	8
54	128	16	32	1
55	256	>128	64	32
56	32	128	64	0.5
57	64	32	16	8
58	64	64	64	8
60	128	128	64	1
61	32	16	64	4
62	128	>128	64	16

Table 2. Results of checkerboard and time-kill studies of imipenem and fosfomycin against methicillin-resistant *Staphylococcus aureus* (MRSA)

Reaction	No. of MRSA strains demonstrating synergy and indifference in the following study :				
	checkerboard	Time-kill			
		3 h	6 h	24 h	48 h
Synergy	14	9	12	14	14
Indifference	3	5	2	0	0

indifference as a change in CFU/ml that was neither greater nor less than a 100-fold change in CFU/ml.

RESULTS

As shown in Table 1, the MICs of methicillin for 50% and 90% of the strains tested were 64 and 256 μg/ml, respectively. The range was 32 to 256 μg/ml. The MICs of oxacillin for 50% and 90% of the strains tested were 64 and >128 μg/ml, respectively. The range was 16 to >128 μg/ml. The MICs of fosfomycin for 50% and 90% of the strains tested were 64 and 64 μg/ml, respectively. The range was 16 to 64 μg/ml. The MICs of imipenem for 50% and 90% of the strains tested were 2 and 16 μg/ml, respectively. The range was 0.5 to 32 μg/ml. As shown in Table 2, of the 17 strains of MRSA included in the checkerboard study, 14 demonstrated synergy and 3 indifference. No antagonism was observed in this study. Time-kill studies revealed synergy in 9 and 12 of the 14 strains tested at 3 and 6 h, respectively, while the remaining 5 and 2 showed indifference at 3
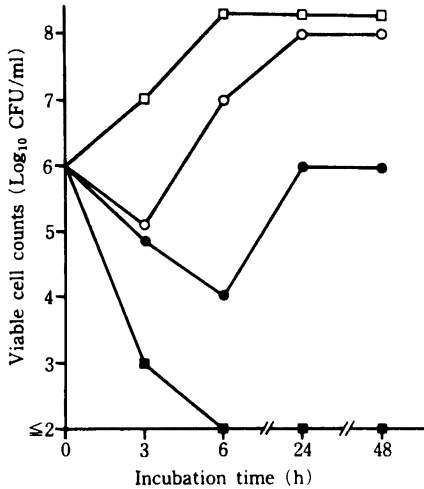
Fig. 1. Time-kill curves of a representative strain (No. 62 strain) of methicillin-resistant *Staphylococcus aureus* showing synergy between imipenem and fosfomycin

● : imipenem 15 μg/ml, ○ : fosfomycin 32 μg/ml,
■ : imipenem 15 μg/ml + fosfomycin 32 μg/ml,
□ : control

and 6 h, respectively. All 14 strains tested revealed synergy at both 24 and 48 h (Table 2). The time-kill curve of a representative strain (No. 62 strain) of MRSA showing synergy between imipenem and fosfomycin is shown in Fig. 1. The MICs of imipenem and fosfomycin against this strain were 16 and 64 μg/ml, respectively, and its FIC index was 0.37.

DISCUSSION

In this study, we investigated the effect of imipenem with fosfomycin against MRSA frequently found in hospitalized patients at the Chiba University Hospital.

Because of its broad spectrum of activity at levels achievable in the blood, imipenem is usually regarded as an antimicrobial agent for use in monotherapy rather than in combination regimens. Hence the practical implications of its *in vitro* interaction with other antimicrobial agents may be inconsequential. Nevertheless, the fact that organisms characteristically tolerant or with a special tendency to develop tolerance to cell-wall -active agents were inhibited and killed synergistically at high rates by particular combinations of imipenem with other antimicrobial agents, at

clinically relevant concentrations, is noteworthy and potentially useful. INOUE et al. have reported that the combinations of imipenem with cefazolin, and imipenem with ceftizoxime demonstrated synergistic inhibitory activity against 80% and 96%, respectively, of 25 MRSA strains tested[31]. In this study, of the 30 strains of MRSA tested, 13 strains were not used because fosfomycin MICs of these strains were outside the range of drug concentrations tested. The combination of imipenem with fosfomycin demonstrated synergistic inhibitory activity against 82.3% of 17 strains of MRSA for which the MIC of fosfomycin was ≤ 64 μg/ml. The time-kill studies also demonstrated that this combination was synergistically active against all 14 strains of MRSA tested. Imipenem alone and fosfomycin alone produced regrowth, but none occured with the combination, even after 48 h. In our *in vitro* study we demonstrated synergistic activity of this combination against a high percentage of MRSA clinical isolates, but further animal and clinical studies are necessary to confirm these results.

References

1) THOMPSON R L, WENZEL R P : Editorial. International recognition of methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. 97 : 925 ~ 926, 1982

2) WENZEL R P : The emergence of methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. 97 : 440 ~ 442, 1982

3) SORRELL T C, PACKHAM D R, SHANKER S, FOLDES M, MUNRO R : Vancomycin therapy for methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. 97 : 344 ~ 350, 1982

4) WATANAKUNAKORN C : Treatment of infections due to methicillin-resistant *Staphylococcus aureus*. Ann. Intern. Med. 97 : 376 ~ 378, 1982

5) FARBER B F, MOELLERING R C : Retrospective study of the toxicity of preparations of vancomycin from 1974 to 1981. Antimicrob. Agents Chemother. 23 : 138 ~ 141, 1983

6) MASUR H, FRANCIOLI P, RUDDY M, MURRAY H W : Vancomycin serum levels and toxicity in chronic hemodialysis patients with *Staphylococcus aureus* bacteremia. Clin. Nephrol. 20 : 85 ~ 88, 1983

7) BRAVENY I : *In vitro* activity of imipenem. A review. Eur. J. Clin. Microbiol. 3 : 456 ~ 462, 1984

8) KROPP H, GORCKENS L, SUNDELOF J G, KAHN F M : Antibacterial activity of imipenem : the first thienamycin antibiotic. Rev. Infect. Dis. 7 (suppl. 3) : 389 ~ 410, 1983

9) AZNAR J, GARCIA IGLESIAS C, PEREA E J : Comparative activity of imipenem (N-formimidoyl-thienamycin) on enterococci and its interactions with aminoglycosides. J. Antimicrob. Chemother. 13 : 129 ~ 132, 1984

10) ELIOPOULOS G M, MOELLERING R C : Susceptibility of entercocci and Listeria monocytogenes to N-formimidoyl thienamycin alone and in combination with an aminoglycoside. Antimicrob. Agents Chemother. 19 : 789 ~ 793, 1981

11) GOMBERT M E, BERKOWITZ L B, CUMMINGS M C : Synergistic effect of N-formimidoyl thienamycin with gentamycin and amikacin against Staphylococcus faecalis. Antimicrob. Agents Chemother. 23 : 245 ~ 247, 1983

12) WATANAKUNAKORN C, TISONE J C : Synergism between N-formimidoyl thienamycin and gentamycin or tobramycin against enterococci. Antimicrob. Agents Chemother. 22 : 1082 ~ 1083, 1982

13) DÍEZ ENCINO M, MATEOS LINDEMANN M L, GUTIÉRREZ ALTÉS A : In vitro evaluation of N-formimidoyl thienamycin (MK 0787) combined with amikacin against gram-negative bacilli and Staphylococcus aureus. Antimicrob. Agents Chemother. 22 : 1064 ~ 1066, 1982

14) HOOTON T M, BLAIR A D, TURCK M, COUNTS G W : Synergism at clinically attainable concentrations of aminoglycoside and β-lactam antibiotics. Antimicrob. Agents Chemother. 26 : 535 ~ 538, 1984

15) KALLICK C, RICE T, NORSEN J, RAJASHEKARAIAH K, MARSH D, MCCULLEY D, THADHANI K : In vitro activity of N-formimidoyl thienamycin against Pseudomonas and staphylococci associated with endocarditis, p. 725 ~ 726. In PERITI P and GIALDRONI GRASSI G (ed.), Current chemotherapy and immunotherapy, Vol. 1. American Society for Microbiology, Washington, D.C., 1982

16) NEU H C, LABTHAVIKUL P : Comparative in vitro activity of N-formimidoyl thienamycin against gram-positive and gram-negative aerobic and anaerobic species and its β-lactamase stability. Antimicrob. Agents Chemother. 21 : 180 ~ 187, 1982

17) REYES M P, LERNER A M : Current problems in the treatment of infective endocarditis due to Pseudomonas aeruginosa. Rev. Infect. Dis. 5 : 314 ~ 321, 1983

18) AMPEL N M, MOON-MCDERMOTT L, KEATING M,

ZINNER S H : In vitro activity of aztreonam in combination with four other antibiotics against gram-negative bacilli and Staphylococcus aureus. J. Antimicrob. Chemother. 13 : 398 ~ 399, 1984

19) BERTRAM M A, YOUNG L S : Imipenem antagonism of the in vitro activity of piperacillin against Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 26 : 272 ~ 274, 1984

20) ZINNER S H, KLASTERSKY J : In vitro activity of N-formimidoyl thienamycin (MK 0787) alone and combined with other β-lactam compounds and gentamycin, p. 728 ~ 730. In PERITI P and GIALDRONI GRASSI G (ed.), Current chemotherapy and immunotherapy, Vol. 1. American Society for Microbiology, Washington, D.C., 1982

21) MILLER M A, FINAN M, YOUSUF M : In vitro antagonism by N-formimidoyl thienamycin and cefoxitin of second and third generation cephalosporins in Aeromonas hydrophila and Serratia marcescens. J. Antimicrob. Chemother. 11 : 311 ~ 318, 1983

22) BROWN T H, ALFORD R H : Antagonism by chloramphenicol of broad-spectrum β-lactam antibiotics against Klebsiella pneumoniae. Antimicrob. Agents Chemother. 25 : 405 ~ 407, 1984

23) KAHN F M, KAHN J S, CASSIDY P J, KROPP H : The mechanism of action of fosfomycin (phosphonomycin). Ann. N.Y. Acad. Sci. 235 : 364 ~ 386, 1974

24) ALVAREZ S, JONES M, BEAK S L : In vitro activity of fosfomycin, alone and in combination, against methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 28 : 689 ~ 690, 1985

25) GUENTHNER S H, WENZEL R P : In vitro activities of teichomycin, fusidic acid, flucloxacillin, fosfomycin, and vancomycin against methicillin-resistant Staphylococcus aureus. Antimicrob. Agents Chemother. 26 : 268 ~ 269, 1984

26) KESTLE D G, KIRBY W M M : Clinical pharmacology and in vitro activity of phosphonomycin. Antimicrob. Agents Chemother. p. 332 ~ 337, 1969

27) NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS : Methods for dilution antimicrobial tests for bacteria that grow aerobically. Approved standard, publication M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa. 1985

28) VAN CAEKENBERGHE D L, BREYSSENS J : In vitro synergistic activity between bismuth subcitrate and various antimicrobial agents against Camp-

ylobacter pyloridis (*C. pylori*). Antimicrob. Agents Chemother. 31：1429～1430, 1987

29) KAWABATA N, SHIRAHATA Y, DOI S, UEMURA K, YAGINURA Y：A study on serum level and urinary excretion of fosfomycin‐Na in man with special reference to pharmacokinetic analysis. Jap. J. Antibiotics 31：549～560, 1978

30) NORRBY S R, ROGERS J D, FERBER F, JONES K H, ZACCHEI A G, WEIDNER L L, DEMETRIADES J L,

GRAVALLESE D A, HSIEH J Y K：Disposition of radiolabeled imipenem and cilastatin in normal human volunteers. Antimicrob. Agents Chemother. 26：707～714, 1984

31) INOUE M, HASHIMOTO H, MATSUI H, SAKURAI N, OHKUBO T：Synergism of imipenem in combination with cefazolin and ceftizoxime against methicillin‐resistant *Staphylococcus aureus*. Chemotherapy 37：869～876, 1989

Methicillin 耐性黄色ブドウ球菌に対する imipenem と fosfomycin との併用効果

高 橋 公 毅・菅 野 治 重

千葉大学医学部附属病院検査部*

宮 崎 瑞 明

千葉大学医学部肺癌研究施設内科

Imipenem（IPM）＋fosfomycin（FOM）の併用効果を臨床材料より分離した methicillin 耐性黄色ブドウ球菌（FOM の MIC は≦64 μg/ml）を用い微量液体希釈法により検討した。相乗作用（FIC index≦0.5）は，供試菌株 17 株中 14 株 82.3%に，不関は 3 株 17.6%に認められた。また IPM＋FOM の組み合わせは，殺菌曲線でも 24 と 48 時間目に 14 株中 14 株に相乗効果が認められ，methicillin 耐性黄色ブドウ球菌感染症に対する臨床的有用性がうかがわれた。

*千葉市亥鼻 1-8-1