Arch. Pharm. Res. Vol.18, No.6, pp. 423-426, 1995

In vitro and *in vivo* Activities of SM-101, a Mixture of Metampicillin and Sulbactam

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(Received September 14, 1995)

SM-101 is a mixture of metampicillin and sulbactam(2 : 1). The antibacterial activities of SM-101 were compared with those of metampicillin, piperacillin and Augmentin. It showed powerful antibacterial activities against major strains. Except *P. aeruginosa* and *S. marcescens*, the *in vitro* antibacterial activity of SM-101 was higher than those of metampicillin, piperacillin and Augmentin against *Staphylococcus* spp., *Streptococcus* spp., *Morganella morganii*, *E. coli*, and *Proteus* spp. The ED_{50} values of SM-101 were two-fold or greater than those of metampicillin, piperacillin and Augmentin against β-lactamase producing strains, *P. mirabilis* GN 79 and *M. morganii* MB4-11. The *in vivo* efficacy of SM-101 was more active than metampicillin and piperacillin and similar to Augmentin against *S. aureus* Smith, *E. coli* MB4-01 and *K. pneumoniae* MB4-02.

Key words : SM-101, Antibacterial activity, Sulbactam, Metampicillin, Lactamase, ED50

INTRODUCTION

Since the discovery of penicillin, there has been a dramatic progression of new antimicrobial agents from medicine and clinical laboratories to successful clinical use. Many kinds of antibiotics showed powerful activities to bacterial infections. However, the emergence of antibiotic resistance is a major factor limiting long term successful use of an antimicrobial agents. Specially, β-lactam antibiotics were so widely used to bacterial infections that resistances were apparently increased. By far the most serious and widespread mechanism of resistance is the presence of a β-lactamases. β-lactamases hydrolyzed the cyclic amide bond in β-lactam molecules, resulting in the loss of their antibacterial activity. A variety of inhibitors have been used to reduce the activity of β-lactamases. Clavulanic acid and sulbactam were well known for β-lactamases inhibitors (Kuck *et al.*, 1988) and they should be examined because of their extensive use for clinical treatment to date. Sulbactam is a weak antibacterial agents its own right and synergy has been shown between sulbactam and several β-lactams including ampicillin, amoxicillin and cefoperazone (Suginaka *et al.*, 1982; Goto *et al.*, 1985; Nakazawa *et al.*, 1982; Chong *et al.*, 1989). Metampicillin and its salt had relatively poor acitivity against clinically isolated resistant bacterial strains. The mixture of metampicillin and sulbactam had a marked increase in inhibition against Gram-positive and Gram-negative bacteria (U.K. patent No. 1,081, 093). In this report, we described antibacterial activities of SM-101, the mixture of sulbactam and metampicillin (USA Pat. No. 5,049,384) (Kim, 1991).

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MATERIALS AND METHODS

Materials

Metampicillin, piperacillin and SM-101 (metampicillin + sulbactam = 2 : 1) were provided by Samsung Pharmaceuticals Inc., Korea; Augmentin (amoxicillin + clavulanate = 2 : 1) was purchased(Il Sung Pharm Co., Ltd.) in Korea.

The major strains in our laboratory were obtained from ATCC (American Type Culture Collection) and clinical isolates from hospitals in Seoul between 1990-1993. *S. aureus* Smith, *P. mirabilis* GN 79 were provided by Professor Goto of Toho University, Japan.

Susceptibility tests

Susceptibility tests of bacterial strains were done by

the agar dilution method with Mueller-Hinton agar (MHA, Difco) by the standard procedure (Japan Society of Chemotherapy, 1989). The MICs against Streptococci and *Haemophilus* were determined with Brain Heart Infusion (BHI, Difco) supplemented with 1% bactosupplement B (Difco) and anaerobic bacteria were grown on fluidthioglycolate medium (FT, Difco) at 37°C. Test compounds were prepared according to manufacturer's instructions and diluted in distilled water. The compounds were diluted to 2 mg/ml in distilled water by serial two-fold dilution and the final drug concentrations was adjusted to 100 μg/ml to 0.025 μg/ml. Agar plates containing test compounds were inoculated with the strains using a microplanter (Sakuma) which delivered 5 μl and yielded a final inoculum of 1×10^3 to 7.5×10^3 cells. Drug-free growth control strains were included. The MIC was defined as the lowest concentration of drug showing no visible growth. β-lactamase testing was carried out with the nitrocefin disc test (cefinase, BBL).

Animal treatments

We selected three pathogenic strains isolated from clinical infections for pathogenecity tests. The effects of SM-101 and the other antibiotics on systemic infections were studied in ICR male mice (age, 4 weeks; weights, 18 to 22 g). Inocula were prepared by diluting an overnight Mueller-Hinton broth (MHB) culture in fresh broth and adding bacteriological mucin (Difco), such that the challenge inocula were equivalent to 100 times the minimal lethal dose (MLD), the number of bacteria required to kill 100% of the control mice under the same condition, all of the untreated mice died within 2 days (Cleeland and Squires, 1991). 0.3 ml volumes of suspension were injected *i.p.* and two groups of animals received mucin and saline as controls. The mice were inoculated *i.v.* in the tail vein with single 0.1 ml volumes of antibiotic at 6 dosage or more, between 1-2 h after infection. The 50% effective dose (ED_{50}) was estimated by the Litchfield-Wilcoxon method (Litchfield and Wilcoxon, 1949) from results of three tests to determine 7 day survival ratios.

RESULTS

The results of the antibacterial activities of SM-101 were presented in Tables I, II, and III. SM-101 showed powerful activity against Gram positive and Gram negative bacteria. Against Gram positive bacteria such as methicillin sensitive *S. aureus* (MSSA), methicillin resistant *S. aureus* (MRSA) and *E. faecalis,* SM-101 was two to 8-fold more active than metampicillin and piperacillin and similar to Augmentin. Against *E. coli, Enterobacter, Proteus* spp. *S. marcescens, M. morganii* and *K. pneumoniae,* SM-101 was two-fold more active than metampicillin and similar to piperacillin and Augmentin. The *in vitro* activity of SM-101 against *P. aeruginosa* was inferior to that of piperacillin. Table III showed the *in vivo* efficacy in systemic infections and ED_{50} values of SM-101, metampicillin, piperacillin and Augmentin against β-lactamase producing strains, *P. mirabilis* GN 79 and *M. morganii* MB4-11. ED_{50} values for SM-101 correlated with *in vitro* MICs showing more potent activity than metampicillin and piperacillin, and similar to Augmentin against *S. aurues* Smith, *E. coli* MB4-01 and *K. pneumoniae* MB4-02. The *in vivo* antibacterial activities of SM-101 was two-fold or greater than those of metampicillin, piperacillin and Augmentin against β-lactamase producing strains.

Table I. Susceptibility of major strains to antibiotics

Strain	MIC (μg/ml)[a]			
	SM-101	Metampicillin	Piperacillin	Augmentin[b]
S. aureus ATCC 6538P	0.39	0.39	0.39	0.78
S. aureus Smith	0.20	0.10	0.78	0.10
E. faecalis ATCC 29212	1.56	6.25	6.25	1.56
S. pneumoniae ATCC 6301	0.78	1.56	0.78	0.20
E. coli ATCC 25922	6.25	25	0.78	1.56
E. coli MB4-01	1.56	1.56	0.39	1.56
K. pneumoniae MB4-02	3.13	12.5	1.56	0.78
C. freundii ATCC 6750	12.5	50	0.78	1.56
E. cloacae ATCC 27058	3.13	3.13	0.20	0.39
P. mirabilis GN79	6.25	100	12.5	12.5
S. marcescens ATCC 27117	25	50	0.78	25
M. morganii MB4-11	12.5	50	12.5	6.25
A. calcoaceticus ATCC 23055	0.39	0.78	0.05	0.10
P. aeruginosa ATCC 25619	1.56	1.56	3.13	3.13
H. influenzae ATCC 33391	0.20	0.78	0.39	0.10
B. fragilis ATCC 29762	3.13	6.25	3.13	1.56

[a]10^6CFU/ml
[b]Augmentin:amoxicillin+clavulanate (2 : 1).

DISCUSSION

Many kinds of antibiotics showed powerful an-

Table II. Susceptibility of clinical isolates against antibiotics

Strain	Drug	MIC (μg/ml)[a]		
		50%	90%	Range
Methicillin-sen-	SM-101	3.13	25	1.56-12.5
sitive	metampicillin	12.5	50<	3.13-50<
S. aureus	piperacillin	25	50	6.25-50
(40)	Augmentin	1.56	6.25	0.10-6.25
	SM-101	25	50<	6.25-50<
Methicillin-resistant	metampicillin	50<	50<	50<
S. aureus	piperacillin	50<	50<	50<
(40)	Augmentin**	12.5	25	0.78-25
	SM-101	1.56	1.56	0.78-3.13
E. faecalis	metampicillin	6.25	12.5	1.56-12.5
(50)	piperacillin	25	25	2.5-50<
	Augmentin	1.56	12.5	0.20-50
	SM-101	6.25	25	1.56-25
E. coli	metampicillin	50<	50<	50<
(46)	piperacillin	12.5	50	6.25-50<
	Augmentin	6.25	50	0.20-50
	SM-101	25	50<	1.56-50
E. cloacae	metampicillin	50<	50<	12.5-50
(38)	piperacillin	3.13	50<	0.39-50
	Augmentin	1.56	25	0.39-50
E. aerogenes	SM-101	12.5	50<	12.5 50<
(30)	metampicillin	25	50<	12.5 50<
	piperacillin	3.13	25	1.56 50
	Augmentin	1.56	25	0.78-50
K. pneumoniae	SM-101	3.13	50	3 13-12.5
(37)	metampicillin	25	50<	12.5-50<
	piperacillin	3.13	50	0.39-12.5
	Augmentin	3.13	25	0.20-25
C. freundii	SM-101	50	50<	50<
(30)	metampicillin	25	50<	12.5-50<
	piperacillin	12.5	50	6.25-50<
	Augmentin	6.25	25	0.78-50
Proteus spp.	SM-101	6.25	25	1.56-50
(48)	metampicillin	50	50<	50≤
	piperacillin	3.13	25	0.20-25
	Augmentin	3.13	25	0.39-25
S. marcescens	SM-101	25	50<	0.025-50
(30)	metampicillin	50<	50<	0.025-50<
	piperacillin	3.13	6.25	0.025-25
	Augmentin	25	50	3.13-50<
M. morganii	SM-101	6.25	25	1.56-50<
(30)	metampicillin	25	50<	1.56-50
	piperacillin	12.5	50	6 25-50
	Augmentin	6.25	12.5	3.13-50
P. aeruginosa	SM-101	50<	50<	12 5-50
(38)	metampicillin	50<	50<	50
	piperacillin	6.25	25	1.56-50
	Augmentin	50	50<	25-50<

[a]10^6CFU/ml
[b]Augmentin : amoxicillin+clavulanate (2 : 1)

Table III. Protecting effects of antibiotics aganist infection

Strain	Drug	Challenge Dose (cells/mouse)	MIC[a] (μg/ml)	ED_{50}[b] (mg/kg/dose)
S. aureus Smith	SM-101	6×10^4	0.20	2.37(0.96-5.86)
	metampicillin		0.10	3.41(1.92-6.08)
	piperacillin		0.78	6.04(2.74-13.32)
	Augmentind		0.10	1.14(0.95-1.35)
E. coli MB4-01	SM-101	6×10^3	1.56	6.27(2.55-15.42)
	metampicillin		1.56	7.34(2.55-21.13)
	piperacillin		0.39	9.40(4.65-18.99)
	Augmentin		1.56	5.20(3.74-7.23)
K. pneumoniae	SM-101	2×10^5	3.13	6.24(2.63-14.83)
MB4-02	metampicillin		12.5	10.23(4.62-22.66)
	piperacillin		1.56	9.82(4.49-21.45)
	P. mirabilisc		0.78	6.08(4.68-7.90)
P. mirabilis[c]	SM-101	2×10^5	6.25	2.66(1.67-6.09)
GN79	metampicillin		100	18.44(7.66-43.98)
	piperacillin		12.5	9.83(4.28-22.66)
	Augmentin		12.5	5.60(2.33-13.4)
M. morganii[c]	SM-101	1×10^6	12.5	3.45(1.51-7.91)
MB4-11	metampicillin		50	4.88(2.16-11.05)
	piperacillin		12.5	5.17(2.30-11.65)
	Augmentin		6.25	3.92(2.48-6.19)

[a]10^6CFU/ml
[b]By Litchfield-Wilcoxon method (95% confidence limits)
[c]β-lactamase producing strains
[d]Augmentin : amoxicillin+clavulanate (2 : 1)

tibacterial activities to bacterial infections, but resistant strains were proportionally increased with the use of antimicrobial agents. Recently β-lactam antibiotics were so widely used to bacterial infections and then their resistance were increased. Although this problem was recognized shortly after the commercial introduction of antimicrobial agents, it seems that resistance is now emerging at more rapid rate than ever before. As a result, there is a need for new compounds; advanced-generation penicillins, cephalosporins, fluoroquinolones and carbapenems etc. Sulbactam and clavulanic acid were well known β-lactamase inhibitors (Kuck *et al.*, 1988) i. e. they bound to β-lactamases (Greenwood and Eley, 1982) and suppressed the enzyme activity. SM-101 is a mixture of metampicillin and sulbactam (2 : 1). We had compared the antibiacterial activities with those of metampicillin, piperacillin and Augmentin. Metampicillin and its salt had relatively poor activity against clinical isolates, but combinations of sulbactam and metampicillin increased their activity in treating bacteriai infections. This mixture showed greater activity than those of metampicillin and piperacillin against major strains. SM-101 also has activity comparable to that of Augmentin against *Staphylococcus* and *Streptococcus*. Against β-lactamase producing strains, *in vivo* activity of SM-101 was two-fold or greater than those of metampicillin, piperacillin and Augmentin. The mechanism of antibacterial activity of SM-101 and Augmentin is partially based on β-lactamases inhibitions. SM-101 appears to be modestly tolerant to β-lactamases despite the relatively low efficiency against Gram negative bacteria. To develop SM-101 into new medicine, it needs further studies about SM-101; PBP binding, enzyme hydrolysis, antagonism, stoichiometry, enzyme hydrolysis, cross-resistance

and pharmacokinetic profiles etc. The antibacterial spectrum of SM-101 resembled that of the reference antibiotic Augmentin. It is thought that SM-101 can be developed as a medicine to control the infections caused by some Gram positive and negative bacteria resistant to amoxicillin, metampicillin and piperacillin.

ACKNOWLEDGEMENT

We gratefully acknowledge that this work was supported by RCNDD, Seoul National University (KOSEF).

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