THERAPEUTIC EFFECT OF SF-2103A, A NOVEL CARBAPENEM ANTIBIOTIC, IN COMBINATION WITH CEFOTAXIME, CEFOPERAZONE AND OTHER CEPHALOSPORINS

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Combinations of SF-2103A with cefotaxime, cefoperazone or cefazolin showed synergistic efficacy at a wide range of combination ratios against experimental infection in mice due to *Proteus vulgaris* GN76/C-1, producing type Ic cephalosporinase, *Escherichia coli* No. 29/36 RGN823, producing type IIIa (TEM-2) penicillinase and *E. coli* GN206, producing type Ib cephalosporinase. These effects by SF-2103A were greater than those seen with sulbactam. The *in vitro* and *in vivo* synergistic activities were roughly correlated. Potent *in vivo* activity of SF-2103A was related to good pharmacokinetic properties, with blood half-life of 30 minutes and urinary recovery of 55.2% after parenteral administration to rats. Furthermore, SF-2103A was stable to rat kidney homogenate. The high stability of SF-2103A in aqueous and biological media was correlated with the sulfonate group at C-3.

SF-2103A, a novel carbapenem antibiotic produced by *Streptomyces sulfonofaciens* SF-2103 showed a powerful inhibitory activity against β-lactamases, in particular chromosomally mediated cephalosporinases of Gram-negative bacteria[1]. The synergistic antibacterial activity of SF-2103A in conjunction with various cephalosporins was demonstrated *in vitro* in the previous paper[2] by reduction of the MIC values, low FIC indices and increased bactericidal activity. In this paper, the antibacterial activity of the combination of SF-2103A with various cephalosporins against experimental infections in mice is described. Pharmacokinetic behaviors of SF-2103A in rodents are also studied to confirm the *in vivo* efficacy.

Materials and Methods

Drugs and Bacterial Strains

SF-2103A was prepared by fermentation in Pharmaceutical & Developmental Laboratories, Meiji Seika Kaisha, Ltd., Kawasaki. Cefminox (MT-141) and sulbactam were synthesized, and clavulanic acid, SF-2050B[3] and MM 4550 were prepared by fermentation in this laboratory. Cefotaxime was from Hoechst Japan Limited, Tokyo, cefoperazone from Toyama Chemical Co., Ltd., Tokyo, ceftizoxime and cefazolin from Fujisawa Pharmaceutical Co., Ltd., Osaka, cefmetazole from Sankyo Co., Ltd., Tokyo, latamoxef from Shionogi & Co., Ltd., Osaka, and cephalothin from Meiji Seika Kaisha, Ltd., Tokyo.

Bacterial strains used for the *in vivo* experiments were stock cultures in this laboratory, and kept at $-20°C$ in 10% skim milk (Difco Laboratories, Detroit) before use. *Proteus vulgaris* GN76/C-1 is a strong producer of chromosomal type Ic cephalosporinase. *Escherichia coli* No. 29/36 RGN823 is a strong producer of R-plasmid mediated type IIIa (TEM-2) penicillinase. *E. coli* GN206 is a weak producer of chromosomal type Ib cephalosporinase[4].

Determination of ED_{50}s

Microorganisms were cultured overnight at 37°C in heart infusion agar (Eiken Chemicals, Tokyo).

Four-week-old male ICR mice weighing 20 to 21 g, eight per group, were injected intraperitoneally with 2 to 77 times the 50% lethal doses of the bacteria, suspended in 0.5 ml of saline containing 2.5~5.0% mucin (Difco Laboratories). One hour later, the mice were given a single subcutaneous injection of serial two-fold-diluted doses of test antibiotics or their combinations. Surviving mice were recorded one week after infection. The 50% effective doses (ED_{50}s, milligram per mouse) and the 95% confidence limits were determined by the LITCHFIELD and WILCOXON method.

To assess the *in vivo* effect of the combination of the two agents, the fractional effective dose (FED) index was used as a substitute for the fractional inhibitory concentration (FIC) index[5]. The *in vivo* synergy was defined by FED index <0.5. A sign of inequality was ignored for the calculation of FED index.

Determination of Blood Levels and Half-lives in Rats

Eight-week-old Wistar rats weighing 290~350 g (male, 3 to 5 in a group) were used. Aqueous solutions of SF-2103A (10 mg/ml) and cefoperazone or cephalothin (20 mg/ml) were co-administered subcutaneously in a volume of 0.29~0.35 ml per rat.

Blood samples were obtained from the rats by cardiac puncture at 5, 15, 30, 60, 120, 180 and 240 minutes after dosing. The blood samples were diluted with phosphate-citrate buffer at pH 7.0 for assays.

SF-2103A was determined by the β-lactamase-inhibitory assay, as described below. The minimum concentration for detection was 0.006 μg/ml. Neither cefoperazone nor cephalothin interfered in the assay because of high dilution of the sample solution. Cefoperazone and cephalothin were determined by the paper disc agar diffusion method using *Bacillus subtilis* ATCC 8193 as a test organism. The minimum concentration for determination was 0.2 μg/ml. SF-2103A showed no activity under the conditions employed. The biological half-lives (T1/2) and area under the curves (AUCs between time zero and time infinity) were calculated by applying a one-compartment model. The significance of the results was examined by analysis of covariance.

For the determination of comparative blood levels of SF-2103A, SF-2050B and ampicillin, three or four ICR male mice were used for each experiment. Bloods per group were pooled, and drug levels were determined by the β-lactamase-inhibitory assay (SF-2103A and SF-2050B) or by the paper disc agar diffusion assay (ampicillin) using *B. subtilis* ATCC 8193.

Urinary Recovery of SF-2103A

Under anesthesia with pentobarbital, the bladders of Wistar rats were cannulated with two polyethylene tubes for successive urine collection (30~60 minutes intervals). One minute before sampling, 1 ml of phosphate-citrate buffer (pH 7.0) was injected through a one tube with an injection syringe, and urine samples were recovered from bladder through the other tube. The samples were diluted with phosphate-citrate buffer for assays.

Determination of β-Lactamase Inhibitor in Biological Fluids

β-Lactamase-inhibitory Assay: A sample solution (25 μl) was added on paper discs each containing 0.4 μg of cephalothin, which were placed on the agar plates containing *B. subtilis* ATCC 8193 and the crude β-lactamase prepared by ultra-sonication of *P. vulgaris* GN76/C-1 cells[2]. The inhibition zone due to cephalothin was proportional to the concentration of an inhibitor that protected the hydrolysis of cephalothin from the β-lactamase.

High Performance Liquid Chromatography (HPLC) Assay: A mixture of sample solution (0.1 ml) and cold ethanol (0.1 ml) was shaken, the precipitated proteins were removed by the refrigerated centrifugation at 3,000 rpm for 5 minutes, and the supernatant (10 μl) was injected to a HPLC column.

The HPLC was carried out using a Waters 6000A pump and reverse phase C18 μBondapak Column (Waters Associates Ltd.) (4×250 mm). SF-2103A was detected at 270 nm by an Hitachi UV spectrometer. The column eluant was based on tetrabutylammonium phosphate (Waters Associates Ltd.) (10 ml) plus 0.1 M potassium dihydrogen phosphate buffer (pH 5.0) (490 ml) plus methanol (235 ml) and pumped at 1 ml/minute at 20°C. Under these conditions, a retention time of SF-2103A was about 8 minutes.

Table 1. Effect of administration schedule on the efficacy of the combinations of SF-2103A or sulbactam with cefotaxime (CTX) in mice infected with *Proteus vulgaris* GN76/C-1.

Drug	Ratio	Administration (sc)	No. of administration (time after challenge)	Dose (mg/mouse)	Survival rate
SF-2103A	Alone		1 (1 hour)	2	0/8
CTX	Alone		1 (1 hour)	2	0/8
SF-2103A+CTX	1:2	Simultaneous	1 (1 hour)	0.75	6/8
SF-2103A+CTX	1:2	Simultaneous	1 (1 hour)	1.5	7/8
SF-2103A+CTX	1:2	Simultaneous	2 (1, 2 hours)	0.75	6/8
SF-2103A+CTX	1:4	Simultaneous	2 (1, 2 hours)	1.25	7/8
SF-2103A+CTX	1:2	Simultaneous	2 (1, 2 hours)	1.5	8/8
SF-2103A+CTX	1:2	Separate	SF-2103A 1 (1 hour) CTX 1 (2 hours)	0.75	0/8
SF-2103A+CTX	1:2	Separate	SF-2103A 1 (1 hour) CTX 1 (2 hours)	1.5	1/8
Sulbactam+CTX	1:2	Simultaneous	1 (1 hour)	1.5	5/8
Sulbactam+CTX	1:2	Simultaneous	2 (1, 2 hours)	1.5	4/8
Sulbactam+CTX	1:2	Separate	Sulbactam 1 (1 hour) CTX 1 (2 hours)	1.5	0/8

Determination of Degradation Half-lives in Tissue Homogenates and Serum

Fresh kidney cortex, lung and liver tissues and blood serum were collected from Wistar rats and ICR mice. Each organ (1 g) was sliced into small pieces and homogenized in 3 ml of 0.05 M phosphate buffer (pH 7.0) with a Teflon Potter-Ervehjem homogenizer for 1~2 minutes. After refrigerated centrifugation at 3,000 rpm for 15 minutes, the supernatant was used for degradation study of the antibiotics.

A mixture of 0.2 ml of the supernatant and 0.8 ml of a solution of the appropriate antibiotic (25 μg/ml in 0.05 M phosphate buffer) was incubated at 37°C. Samples were removed at time 0 and at frequent intervals during incubation, and diluted with phosphate buffer for assays.

For degradation study in serum, a mixture of the fresh serum (0.9 ml) and a solution of the antibiotic (0.1 ml, 200 μg/ml in 0.2 M phosphate buffer at pH 7.0) was incubated at 37°C. Fresh serum other than those of rats and mice were obtained from Golden Syrian hamsters, guinea pigs, Beagle dogs, pigs (Yorkshire), Albino rabbits, monkeys (*Macaca irus*) and human (Japanese).

Results

Effect of Combination of SF-2103A with Cefotaxime or Cefoperazone against *P. vulgaris* GN76/C-1

A preliminary experiment was set up to establish the most effective dosing of two agents. Table 1 shows the effect of administration schedule in combination use of SF-2103A and cefotaxime. The test pathogen was *P. vulgaris* GN76/C-1, which is a strong producer of type Ic cephalosporinase. Under the conditions that administration of either SF-2103A or cefotaxime alone was not effective, simultaneous administration of both agents combined in a ratio of 1:2 was found effective when given in a single dose or divided into two equal doses, given with an interval of 1 hour. There was no essential difference between the two routes of administration, as long as the two agents were given simultaneously. However, separate administration of the two agents with an interval of 1 hour resulted in the loss of the *in vivo* activity. The same tendency was observed for the combination of sulbactam and cefotaxime. Accordingly, a bolus administration of the combination 1 hour after the challenge was used throughout the following *in vivo* experiments.

Table 2. *In vivo* effect of SF-2103A and sulbactam in combination with cefotaxime (CTX) or cefoperazone (CPZ) against mice infected with *Proteus vulgaris* GN76/C-1*.

Drug	Ratio	Challenge dose (cfu/mouse)	ED_{50} (mg/mouse) (95% confidence limit)	FED	MIC (μg/ml)
SF-2103A	Alone	2.7×10^7	>2		25
Sulbactam	Alone	8.5×10^7	>8		50
CTX	Alone	8.5×10^7	>8		400
CPZ	Alone	2.7×10^7	>8		1,600
SF-2103A+CTX	1:2	8.5×10^7	0.23 (0.11~0.49)	0.04	0.20
	1:4		0.20 (0.11~0.36)	0.03	
	1:8		0.21 (0.13~0.34)	0.03	
SF-2103A+CTX	1:8	1.0×10^8	0.74 (0.49~1.12)	0.10	
	1:32		0.73 (0.39~1.42)	0.09	
	1:64		*ca.* 0.51		
	1:128		0.61 (0.37~1.00)	0.08	
Sulbactam+CTX	1:2	8.5×10^7	0.68 (0.58~0.80)	0.09	0.39
	1:4		0.98 (0.68~1.40)	0.12	
	1:8		*ca.* 0.84		
Sulbactam+CTX	1:8	1.0×10^8	2.71 (1.78~4.12)	0.34	
	1:32		*ca.* 3.38		
	1:64		3.25 (1.99~5.30)	0.41	
	1:128		*ca.* 6.0	0.75	
SF-2103A+CPZ	1:2	2.7×10^7	0.82 (0.42~1.60)	0.14	0.78
	1:4		0.42 (0.20~0.89)	0.06	0.78
	1:8		0.56 (0.24~1.30)	0.08	0.78

* A producer of type Ic cephalosporinase.

Table 2 summarizes the *in vivo* efficacy of the combinations of SF-2103A or sulbactam with cefotaxime or cefoperazone at various dose ratios against *P. vulgaris* GN76/C-1. While neither SF-2103A nor cefotaxime was effective as a single agent, their combinations showed marked *in vivo* synergy with FED <0.1. The ED_{50} values of CTX were reduced more than 35 times by the 1:2 combination. The ED_{50} values appeared constant over a wide range of combination ratios from 1:2 to 1:128, implying that the addition of SF-2103A to cefotaxime in less than 1% of the component brought about a maximum effect on the *in vivo* activity of the latter.

The combination of sulbactam with cefotaxime also showed marked increase of the *in vivo* activity. However, judging from the FED indices, the effect was three to four-fold inferior to that of SF-2103A. Furthermore, the ED_{50} values involving sulbactam tended to rise as the combination ratio increased, in particular at 1:128, indicating loss of the synergistic activity.

The combination of SF-2103A with cefoperazone also showed marked *in vivo* synergy showing FED of 0.14, although cefoperazone alone was not effective. The ED_{50} values and FED indices did not change by changing the ratio from 1:2 to 1:8. These *in vivo* results were roughly those expected from the synergistic MIC values.

Effect of the Combinations of SF-2103A with Cefoperazone or Cefazolin against *E. coli* No. 29/36 RGN823 and *E. coli* GN206

Table 3 shows the therapeutic effect of the combinations of SF-2103A with cefoperazone or cefazolin against two *E. coli* infections in mice. In contrast to the *P. vulgaris* strain, *E. coli* No. 29/36 RGN823 producing type IIIa (TEM-2) penicillinase was susceptible to SF-2103A, and a single treatment showed ED_{50} values comparable to those of the combination. However, the combinations of SF-

Table 3. *In vivo* effect of SF-2103A and sulbactam in combination with cefoperazone (CPZ) against mice infected with *Escherichia coli* No. 29/36 RGN823[a] or with cefazolin (CEZ) against mice infected with *E. coli* GN206[b].

Drug	Ratio	Challenge dose (cfu/mouse)	ED_{50} (mg/mouse) (95% confidence limit)	FED	MIC (μg/ml)
E. coli No. 29/36 RGN823					
SF-2103A	Alone	6.2×10^6	0.59 (0.38~0.91)		3.13
Sulbactam	Alone	6.2×10^6	>4		50
CPZ	Alone	6.2×10^6	2.6 (1.7~3.9)		1,600
SF-2103A+CPZ	1:2	6.2×10^6	0.86 (0.57~1.3)	0.71	3.13
	1:4		0.70 (0.45~1.1)	0.45	
	1:8		0.52 (0.31~0.88)	0.27	
Sulbactam+CPZ	1:2	6.2×10^6	1.8 (1.1~2.9)	0.61	25
	1:4		1.0 (0.60~1.7)	0.36	
	1:8		1.2 (0.68~2.1)	0.44	
E. coli GN206					
SF-2103A	Alone	1.3×10^6	>4		3.13
Sulbactam	Alone		>8		50
CEZ	Alone		>8		200
SF-2103A+CEZ	1:2	3.1×10^5	2.50 (1.40~4.50)	0.42	
	1:4		0.88 (0.51~1.50)	0.13	
	1:8		1.50 (1.00~2.20)	0.21	
SF-2103A+CEZ	1:8	1.3×10^6	1.26 (0.66~1.90)	0.18	
	1:32		1.42 (0.76~2.64)	0.18	
	1:64		1.44 (0.76~2.74)	0.18	
	1:128		1.91 (1.22~2.98)	0.24	
Sulbactam+CEZ	1:8	1.3×10^6	3.62 (2.29~5.72)	0.45	
	1:32		>4.13		
	1:64		5.08 (3.19~8.08)	0.63	
	1:128		*ca.* 8.06	1.0	

[a] A producer of type IIIa penicillinase.
[b] A producer of type Ib cephalosporinase.

2103A with cefoperazone showed synergistic ED_{50} values at 1:4 and 1:8 ratios. Sulbactam showed a similar *in vivo* synergy in combination with cefoperazone, giving ED_{50} values comparable to those of the SF-2103A combinations, in spite of inferior MIC value.

E. coli GN206 is a weak producer of type Ib cephalosporinase. While SF-2103A, cefazolin or sulbactam were not effective in mice infected with this strain, the combinations of SF-2103A with cefazolin in ratios from 1:2 to 1:128 were effective, giving rise to almost constant ED_{50} values and a synergistic FED index of 0.2. The combination of sulbactam with cefazolin showed similar *in vivo* synergy, but the ED_{50} values were three to four-fold larger than those seen with the combinations of SF-2103A, which is a significant difference. The FED indices of the combinations of sulbactam tended to increase from 0.45 to 1.0 when the ratio was changed from 1:8 to 1:128.

Attempted Correlation of *In Vitro* and *In Vivo* Synergies of Combinations of SF-2103A with Various Cephalosporins

Table 4 shows the ED_{50} values of the 1:2 combinations of SF-2103A with eight cephalosporins against two Gram-negative bacteria producing chromosomal cephalosporinases.

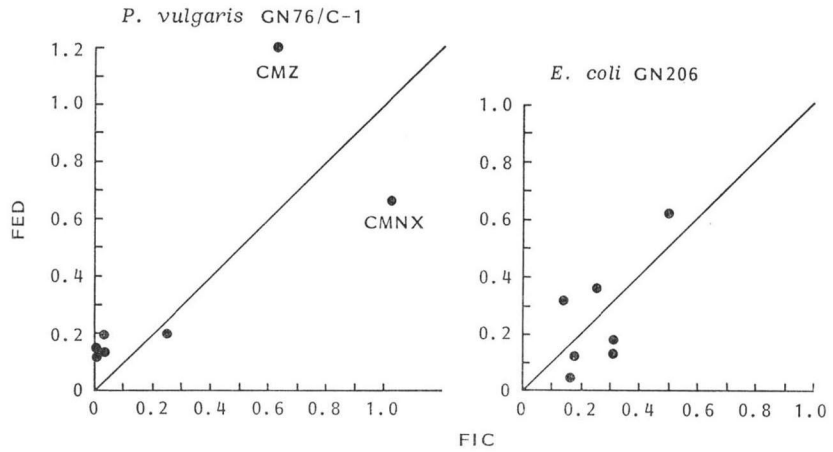
Against *P. vulgaris* GN76/C-1, ceftizoxime in combination with SF-2103A showed potent *in vivo* activity, reflecting the high *in vitro* activity of the combination. Cefotaxime, cefoperazone, cephalothin

Table 4. *In vivo* effect of SF-2103A in 1:2 combinations with eight cephalosporins against mice infected with *Proteus vulgaris* GN76/C-1[a] and *Escherichia coli* GN206[b].

Cephalosporin	ED_{50} (mg/mouse) (95% confidence limit)		MIC (μg/ml)	
	Alone	SF-2103A+β-lactam (1:2)	Alone	SF-2103A+β-lactam (1:2)
P. vulgaris GN76/C-1				
Cefotaxime	>16	0.31 (0.15~0.62)	400	0.20
Cefoperazone[c]	>8	0.82 (0.42~1.6)	>1,600	1.56
Ceftizoxime	1.0 (0.79~1.27)	<0.19	6.25	0.10
Latamoxef	0.70 (0.37~1.31)	0.95 (0.53~1.70)	0.78	
Cefminox	*ca.* 0.06	0.063 (0.04~0.10)	0.78	1.56
Cefmetazole	1.0 (0.43~2.31)	1.75 (1.07~2.85)	50	1.56
Cefazolin	>16	1.62 (0.94~2.80)	>1,600	12.5
Cephalothin	>16	2.39 (1.24~4.61)	>3,200	12.5
E. coli GN206				
Cefotaxime	0.79 (0.47~1.33)	*ca.* 0.125	6.25	1.56
Cefoperazone	2.15 (0.93~4.97)	0.42 (0.23~0.75)	25	0.39
Ceftizoxime	>2.0	0.10 (0.07~0.14)	25	0.78
Latamoxef	0.17 (0.09~0.33)	0.02 (0.01~0.04)	0.78	
Cefminox	0.46 (0.27~0.78)	0.07 (0.05~0.11)	50	6.25
Cefmetazole	2.5 (1.17~5.35)	0.18 (0.10~0.34)	25	3.13
Cefazolin	>8	1.0 (0.56~1.79)	>800	6.25
Cephalothin	>8	2.0 (1.0~4.02)	>3,200	6.25

[a] Challenge dose: 8×10^7 cfu/mouse in 5% mucin.
[b] Challenge dose: 2.5×10^8 cfu/mouse in 2.5% mucin.
[c] Challenge dose: 2.7×10^7 cfu/mouse in 5% mucin.

Fig. 1. Correlation between FIC indices and FED indices of combinations of SF-2103A with seven cephalosporins against two Gram-negative bacteria.
CMZ; cefmetazole, CMNX; cefminox.



and cefazolin also showed synergistic activity *in vivo* when combined with SF-2103A. In contrast, latamoxef, cefminox and cefmetazole in combination with SF-2103A showed the same or lower activity as those they were as single agents, indicating additive effect (FED index, 0.67~1.32).

Against *E. coli* GN206, latamoxef in combination with SF-2103A showed the highest *in vivo* activity, probably reflecting the high activity of the former. Except for cephalothin, all of the tested

cephalosporins showed synergistic *in vivo* activities when combined with SF-2103A, FED index being 0.05~0.28.

Fig. 1 shows the relation between FED and FIC indices for the combinations of SF-2103A with the seven cephalosporins. There was a rough correlation between the two parameters against test pathogens, with the exception for the cefminox and cefmetazole combinations against the *P. vulgaris* infection.

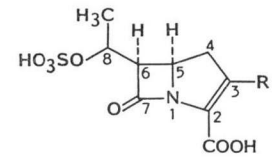
Pharmacokinetics of 1: 2 Combinations of SF-2103A with Cefoperazone and Cephalothin

Table 5 shows a preliminary comparison of the blood levels in mice of SF-2103A, another carbapenem antibiotic (SF-2050B[3]) which possesses the *N*-acetylated aminoethenylthio side chain at C-3, and ampicillin. SF-2103A showed

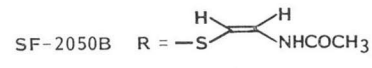
Table 5. Comparative blood levels in mice of SF-2103A, SF-2050B and ampicillin after a subcutaneous dose of 80 mg/kg.

Drug	Blood level (μg/ml)	
	5 minutes	15 minutes
SF-2103A (n=4)	43.2	39.4
SF-2050B (n=3)	0.6	0.3
Ampicillin (n=3)	58.0	99.0

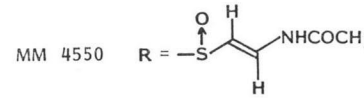
Chart 1. Chemical structures of SF-2103A, SF-2050B and MM 4550.



SF-2103A R = $-SO_3H$



SF-2050B



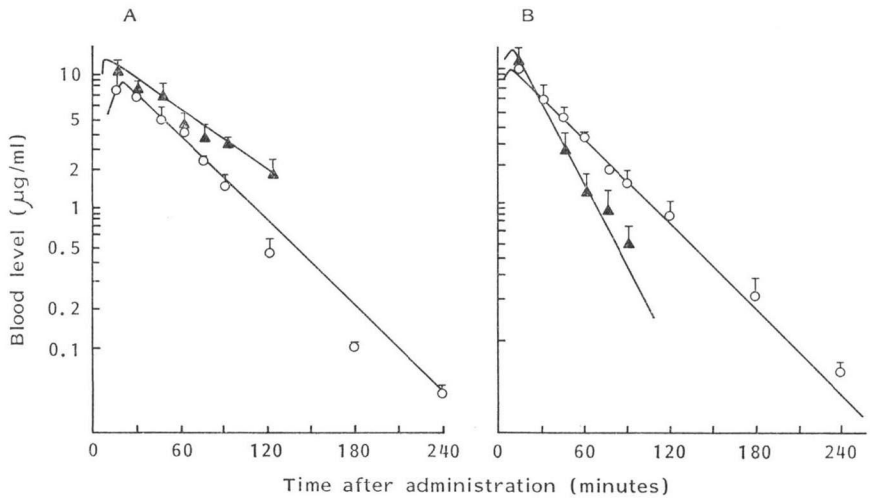
MM 4550

Fig. 2. Blood levels of SF-2103A and cefoperazone (A) or cephalothin (B) co-administered subcutaneously in rats at a dose of 10 mg/kg for SF-2103A and 20 mg/kg for cefoperazone (CPZ) and cephalothin (CET).

Bars indicate standard deviations (n=4).

A) ○: SF-2103A, T1/2: 23.3±2.1 minutes, AUC; 453 μg·minutes/ml,
▲: CPZ, T1/2; 44.8±2.1 minutes, AUC; 758 μg·minutes/ml.

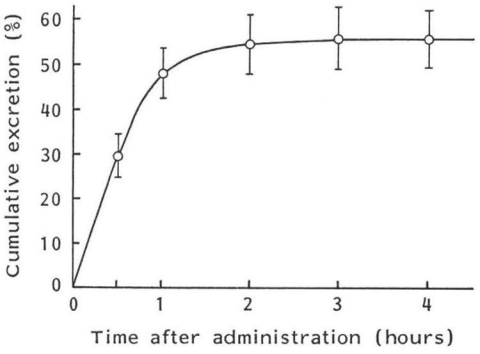
B) ○: SF-2103A, T1/2; 28.0±1.7 minutes, AUC; 508 μg·minutes/ml,
▲: CET, T1/2; 15.0±1.5 minutes, AUC; 370 μg·minutes/ml.



in comparison with ampicillin reasonable blood levels 5 and 15 minutes after being injected subcutaneously. Only very low concentrations of SF-2050B were detected.

Fig. 2 shows blood levels in rats of SF-2103A and cefoperazone (A) or cephalothin (B) after subcutaneous administration of the 1:2 combinations. SF-2103A showed an intermediate half-life (23~28 minutes) between those of cefoperazone and cephalothin. The half-life of SF-2103A after being administered alone (29±3.7 minutes sc; 28.3±3.7 minutes iv) was similar to that found when it was given in the combinations. Thus, the co-administration of SF-2103A with cephalosporins had essentially no effect upon its pharmacokinetics.

Fig. 3. Urinary excretion of SF-2103A in rats after subcutaneous dosing of 10 mg/kg.
Bars indicate standard deviations (n=4).



Absorption of SF-2103A after oral administration (10 mg/kg) in rats was poor, 2.9±0.5%, relative to that after subcutaneous administration.

The urinary excretion of SF-2103A in rats is shown in Fig. 3. On an average a 55.2% recovery was obtained in 4 hours after a single subcutaneous administration. The excretion half-life (29.0±1.8 minutes) was close to the elimination half-life calculated from blood.

Stability of SF-2103A in Aqueous Solution, Tissue Homogenates and Serum

Table 6 shows degradation half-lives of SF-2103A, clavulanic acid and MM 4550 in aqueous solution at various pH. SF-2103A showed comparable stability to clavulanic acid at pH 5 in 5°C, and was more stable than clavulanic acid and MM 4550 at pH 7 and 8.

Table 7 shows the stability of SF-2103A and MM 4550 in tissue homogenates of rats and mice and serum of different animals. While MM 4550 showed markedly reduced stability against kidney homogenate, SF-2103A showed comparable stability in kidney, lung and liver homogenates as well as in serum of rats and mice. It was also rather stable in dog serum. However, the stability of SF-2103A in serum of other animals and of human was decreased. The consistency in the degradation

Table 6. Stability of SF-2103A, clavulanic acid and MM 4550 in 0.2 M phosphate-citrate buffer.

Temperature (°C)	pH	Degradation half-life (hours)		
		SF-2103A (20)*	Clavulanic acid (40)*	MM 4550 (20)*
5	5.0	220	174	158
	7.0	205	136	114
	8.0	157	90.3	79.2
25	5.0	103	111	35.3
	7.0	113	50.7	53.1
	8.0	84.4	25.5	13.6
37	5.0	14.5	19.9	12.3
	7.0	33.0	12.1	8.6
	8.0	13.1	8.2	4.3

* Initial drug concentration (μg/ml). Concentration was measured for each antibiotic by bioassay.

Table 7. Stability of SF-2103A and MM 4550 in tissue homogenates and serum of various animals.

Tissue homogenate and serum		Animal	Degradation half-life (hours) at 37°C		
			SF-2103A*		MM 4550*
			Bioassay	HPLC	Bioassay
Kidney homogenate	(5%, pH 7.0)	Mice	2.0		0.3
		Rats	4.3		0.4
Lung homogenate	(5%, pH 7.0)	Rats	4.7		1.5
Liver homogenate	(5%, pH 7.0)	Rats	8.8		3.2
Serum	(pH 7.35)	Mice	7.1	6.7	1.0
		Rats	3.9	4.1	0.7
Serum	(pH 7.0)	Hamsters	2.2	2.0	
		Guinea pigs	2.7	2.3	
		Dogs	9.3	4.7	
		Pigs		3.6	
		Rabbits	2.1	2.3	
		Monkeys	1.7	1.8	
		Human	0.4		

* Initial concentration of SF-2103A and MM 4550, 20 μg/ml.

half-lives determined by bioassay and HPLC assay except for dogs indicated that SF-2103A was the only active principle in the biological media.

Discussion

Since many of the organisms producing β-lactamases were weakly pathogenic to mice, the strains available for *in vivo* experiments were rather limited. However, the therapeutic efficacy of SF-2103A in combination with eight cephalosporins was demonstrated against three Gram-negative organisms. The ED_{50} values shown by the SF-2103A combinations were generally two to four-fold lower than those of the corresponding combinations with sulbactam. Most notable is the high *in vivo* activity even at a combination ratio of 1: 128, suggesting a potent β-lactamase-inhibitory property of SF-2103A.

Although SF-2103A itself showed moderate *in vitro* activity, the agent responsible for the *in vivo* antibacterial activity of the combinations is mainly the cephalosporin partner, since changes of the inhibitor: cephalosporin ratio of the combinations did not greatly affect their activity. This is in accord with the *in vitro* studies reported in the preceding paper[2].

The high synergistic activity of SF-2103A can, at least partly, be ascribed to its good pharmacokinetic properties. While many of the carbapenem antibiotics gave low blood levels with rapid elimination[6], SF-2103A gives blood levels and half-life comparable to those of the cephalosporin partners. Moreover, it showed a reasonable urinary recovery, which could be correlated with the stability against the kidney homogenate. This suggests that SF-2103A may be rather stable to the renal dihydropeptidase which has been reported to decompose other carbapenem antibiotics, giving a low urinary recovery of them[7~10].

In this connection, it is notable that SF-2103A differs from other carbapenem antibiotics in that it contains the sulfonate group at C-3, which may be responsible for the fair stability of the compound in aqueous solution, serum and tissue homogenates. The sulfate ester group at C-8 may contribute to the β-lactamase inhibitory activity, since sulfate esters were more inhibitory than the corresponding hydroxy compounds in the olivanic acids[6] and C-19393 series[11].

As far as the authors are aware of, there has been no previous report on the *in vivo* efficacy of carbapenem antibiotics as β-lactamase inhibitors. This work appears to be the first examples of *in vivo* synergy between a carbapenem antibiotic and cephalosporins.

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