NOCARDICIN A, A NEW MONOCYCLIC β-LACTAM ANTIBIOTIC. V

IN VIVO EVALUATION

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Nocardicin A is a new monocyclic β-lactam antibiotic which provides a potent therapeutic effect in mice experimentally infected with gram-negative bacilli. When given subcutaneously to mice, the therapeutic effect of the drug was stronger than had been anticipated from *in vitro* studies. Nocardicin A was more potent in therapeutic effect than carbenicillin against infections due to *Pseudomonas aeruginosa, Proteus mirabilis, Pr. vulgaris, Pr. rettgeri* and *Pr. inconstans*, and was similar in effect to carbenicillin against infections due to *Escherichia coli* in mice. In addition, nocardicin A proved to be active against infections due to *Serratia marcescens* and other organisms resistant to β-lactam antibiotics. When nocardicin A was given subcutaneously to mice, blood and hepatic levels of the drug were higher than those of carbenicillin.

Nocardicin A is a new monocyclic β-lactam antibiotic which is active *in vitro* against gram-negative organisms especially *Pseudomonas aeruginosa*, the *Proteus* and *Neisseria* groups.[1] However, the *in vitro* antimicrobial activity of nocardicin A against *Ps. aeruginosa* and *Proteus mirabilis* was markedly influenced by the assay media used.[1,2] Nocardicin A was shown to act synergistically *in vitro* with serum-bactericidal factors against *Ps. aeruginosa* and with polymorphonuclear leukocytes against *Ps. aeruginosa*, *Escherichia coli* and *Pr. vulgaris*. Data are presented in this paper on the therapeutic efficacy of nocardicin A against experimental infections in mice and on the relationship between the drug's *in vitro* and *in vivo* activities.

Materials and Methods

1. Test antibiotics

Antibiotics used were nocardicin A (Fujisawa Research Laboratories), carbenicillin (CBPC, Beecham Research Laboratories), cefazolin (CEZ, Fujisawa Pharmaceutical Co., Ltd.), kanamycin (KM, Meiji Seika Kaisha, Ltd.), nalidixic acid (NA, Daiichi Seiyaku Co., Ltd.), ampicillin (ABPC, Beecham Research Laboratories) and gentamicin (GM, Schering Co., Ltd.).

2. Test organisms

The fresh clinical isolates used were obtained from several hospitals in Japan.

3. *In vitro* antimicrobial activity

The MICs of the test antibiotics were determined by the method described in our previous paper.[1] Unless otherwise specified, for the determination of the MIC, antibiotic medium No. 5 (A-No.5) was used for the *Proteus* group and *Serratia marcescens*, and antibiotic medium No. 3 (A-No.3) for the other organisms.

4. Therapeutic effect on experimental infection in mice

Male ICR-strain mice, aged 4 weeks, weighing 22~24 g or 24~26 g were used in groups of 10 mice each. Each challenge organism was cultured overnight on trypticase soy agar at 37°C and was suspended in 5% bacteriological mucin (Nutritional Biochemical Co.) at conventional inoculum sizes. A cell suspension of 0.5 ml was injected intraperitoneally and the test antibiotics were given subcutaneously in single doses one hour after challenge. The mice were observed for 7 days. The therapeutic effect of the test antibiotics was expressed in terms of ED_{50} (mg/kg) values which were calculated by the Probit method.

5. Determination of viable cell count in the organs of infected mice

Male ICR-strain mice, aged 4 weeks, weighing 22~24 g were used in groups of 4 mice each. An inoculum size of 3.2×10^6 cells of *Pr. mirabilis* suspended in 5% mucin was injected intraperitoneally. Nocardicin A or carbenicillin was injected subcutaneously in a single dose of 348 mg/kg, 87 mg/kg or 22 mg/kg one hour after challenge. The mice were bled to death 9 hours after dosing. The liver and kidneys were removed and homogenized in an appropriate volume of sterile saline solution and the homogenates were suitably diluted in sterile saline solution. To one ml of the aliquots placed in petri dishes, 10 ml of melted brain heart infusion agar was added and mixed well. After overnight incubation at 37°C, the viable cells were counted.

6. Serum levels, tissue levels and urinary excretion in mice

Male ICR-strain mice, aged 6 weeks, weighing 28~32 g were used in groups of 10 or 4 mice each. Nocardicin A or carbenicillin was injected subcutaneously in single doses of 40 or 80 mg/kg. The serum, liver and kidneys were removed after a specified period. Each organ was homogenized with three volumes of M/15 phosphate buffer (pH 7.0). The homogenates were centrifuged at 10,000 r.p.m. for 20 minutes, and the supernatants were used for bioassay. Urinary excretion of nocardicin A and carbenicillin were investigated as follows: Each antibiotic was given subcutaneously to one group of 10 mice in single doses of 40 mg/kg. Each mouse was housed in a metabolism cage for separate collection of urine and faeces. The urine samples were collected over a period of 24 hours following dosing. The concentrations of nocardicin A and carbenicillin were determined by the cylinder-plate method on A-No.3 agar using *Alcaligenes faecalis* 773-9 and *Ps. aeruginosa* NCTC-10490 respectively.

Results

1. Therapeutic Effect of Nocardicin A in Mice Infected with Gram-negative and Gram-positive Bacteria

The therapeutic effect of nocardicin A after a single subcutaneous administration was compared with that of carbenicillin in mice experimentally infected with *Ps. aeruginosa*, the *Proteus* group, *S. marcescens* and *E. coli* (Table 1). Nocardicin A was considerably more active than carbenicillin against infections due to *Ps. aeruginosa*, *Pr. mirabilis*, *Pr. vulgaris*, *Pr. rettgeri*, *Pr. inconstans* and one strain of *S. marcescens*. The activity of nocardicin A was similar to that of carbenicillin against *E. coli* infections. Against *Pr. morganii* infections, nocardicin A was less effective than carbenicillin. The therapeutic activity of nocardicin A in mice infected with other organisms is shown in Table 2. Nocardicin A was ineffective in infections due to all of the bacteria tested.

2. Protecting Effect of Nocardicin A in Mice Infected with Organisms Resistant to Carbenicillin, Ampicillin and Cefazolin

The therapeutic efficacy of nocardicin A in mice infected with *Ps. aeruginosa* and the *Proteus* groups resistant to any or all of the test drugs was investigated. Nocardicin A provided satisfactory therapeutic efficacy against all the infections caused by these organisms (Table 3). These results indicate that no cross-resistance between nocardicin A and other β-lactam antibiotics was seen in

Table 1. Protecting effect of nocardicin A and carbenicillin in mice infected with *Ps. aeruginosa, Proteus* spp., *S. marcescens* and *E. coli*

Organism		Challenge cells/mouse	LD_{50}	ED_{50} (mg/kg)		MIC (μg/ml), 10^6/ml	
				Nocardicin A	Carbenicillin	Nocardicin A	Carbenicillin
Ps. aeruginosa	704	5.8×10^4	20	11.1	188.0	25	200
	708	1.2×10^5	40	2.6	14.1	12.5	12.5
	720	1.0×10^6	10	7.6	378.2	25	50
Pr. mirabilis	503	9.0×10^5	20	10.7	30.7	50	0.78
	504	1.2×10^5	500	21.6	167.6	12.5	0.78
	545	1.8×10^7	200	13.9	61.5	12.5	1.56
Pr. vulgaris	626	8.7×10^5	10	7.8	42.9	200	50
	629	7.4×10^5	200	9.6	117.1	12.5	1.56
Pr. rettgeri	681	8.6×10^4	10	2.4	>851.0	12.5	>400
	683	1.3×10^6	10	4.2	>889.0	3.13	>400
Pr. inconstans	6	1.4×10^8	50	9.8	394.0	3.13	0.39
	21	1.3×10^7	40	6.7	54.0	3.13	0.78
Pr. morganii	673	1.2×10^6	100	134.9	23.4	100	0.78
	6701	6.8×10^5	200	122.0	43.9	100	0.78
S. marcescens	112	6.0×10^4	15	18.2	>952	25	>400
	9905	2.2×10^3	10	20.0	15.9	25	12.5
E. coli	312	6.7×10^4	10	96.0	48.5	100	12.5
	324	1.0×10^5	83	37.8	37.8	100	3.13

Table 2. Protecting effect of nocardicin A, ampicillin (ABPC) and cefazolin (CEZ) in mice infected with *K. pneumoniae*, *Enterobacter* spp., *C. freundii* and gram-positive bacteria

Organism		Challenge cell/mouse	LD_{50}	ED_{50} (mg/kg)			MIC (μg/ml), 10^6/ml		
				Nocardicin A	ABPC	CEZ	Nocardicin A	ABPC	CEZ
K. pneumoniae	130	6.5×10^5	20	423.0	109.8	18.5	400	25	6.25
	282	6.6×10^6	30	85.1	740.0	17.6	100	50	1.56
E. aerogenes	951	5.1×10^7	10	571.0	571.0	>816.0	200	200	100
E. cloacae	921	1.1×10^6	100	>833.0	>833.0	>833.0	400	>400	>400
C. freundii	809	7.4×10^4	10	597.0	531.0	>851.0	>400	>400	>400
S. aureus	2005	3.8×10^8	10	>4900.0	400.0	N.D.*	>400	6.25	N.D.
S. pyogenes	S-23	1.0×10^7	15	>870.0	0.4	N.D.	400	0.1	N.D.
S. pneumoniae	III	5.0×10^6	10	>870.0	26.1	N.D.	200	0.1	N.D.

* not done

in vivo studies, as was the case with *in vitro* activity reported in our previous paper.[1]

3. Bactericidal Effect of Nocardicin A on Viable Cells in Liver and Kidneys of Mice Infected with *Pr. mirabilis*

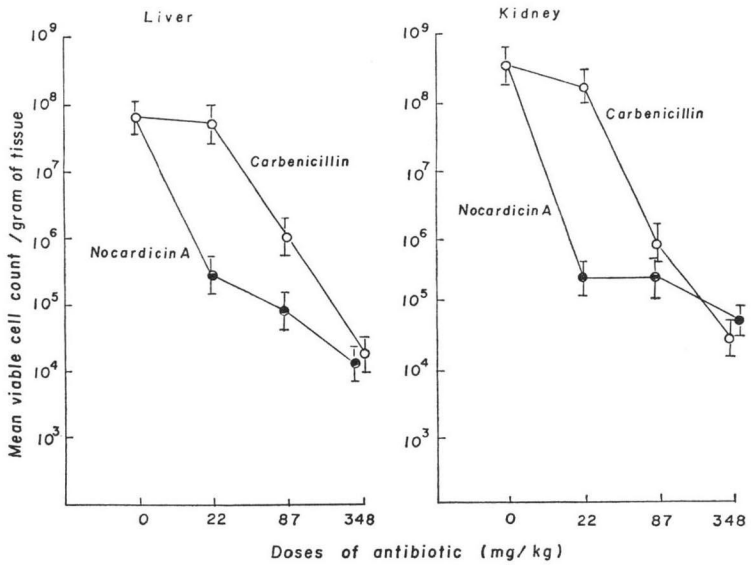
Nocardicin A or carbenicillin was injected subcutaneously in a single dose to mice one hour after challenge. The viable cell count in the liver and kidneys 9 hours after treatment with nocardicin A was compared with that after treatment with carbenicillin. As shown in Fig. 1, the decrease of

Table 3. Protecting effect of nocardicin A against *Ps. aeruginosa* and *Proteus* groups resistant to carbenicillin (CBPC), ampicillin (ABPC) or cefazolin (CEZ)

Organism		Challenge cells/ mouse	×MLD or ×LD_{50}	ED_{50} (mg/kg)				MIC (μg/ml), 10^6/ml			
				Nocardicin	CBPC	ABPC	CEZ	Nocardicin	CBPC	ABPC	CEZ
Ps. aeruginosa	704	5.8×10^4	100	13.0	221.0	N.D.*	N.D.	12.5	200	N.D.	N.D.
Pr. mirabilis	105	8.2×10^7	1 MLD	21.7	>784.0	>784.0	12.5	12.5	>400	>400	3.13
Pr. vulgaris	63	1.0×10^7	1 MLD	3.1	41.4	62.0	41.5	50	50	400	50
Pr. rettgeri	683	1.3×10^7	243	5.9	>851.0	>851.0	N.D.	3.13	400	400	N.D.
Pr. inconstans	6	1.4×10^8	25	10.7	623.0	>930.0	>930.0	6.25	1.56	200	>800

* not done

Fig. 1. Bactericidal effect of nocardicin A and carbenicillin on viable cells in liver and kidneys of mice infected with *Pr. mirabilis* 563.
Treatment: single dose, subcutaneous, 1 hour after challenge. Cell count: 9 hours after treatment



viable cells in the liver and kidneys of infected mice after subcutaneous injection of nocardicin A was more marked than that after carbenicillin treatment. This tendency was especially evident in both the groups receiving low doses (22 mg/kg) of the test drugs, *i.e.* with carbenicillin, the viable cell counts in the liver scarcely decreased, but with nocardicin A the cell counts decreased from 1×10^8/g to 6×10^5/g. Similar findings were obtained in the kidneys.

4. Correlation between *in vitro* Antimicrobial Activity and Therapeutic Effect of Nocardicin A

The *in vitro* activities in HI medium, A-No.3 medium or A-No.5 medium that most closely reflected therapeutic activities were studied for the strains of *Ps. aeruginosa* and *Pr. mirabilis*. As shown in Table 4, nocardicin A has no *in vitro* activity (MICs: >800 μg/ml) against the 4 strains of *Ps. aeruginosa* tested in HI medium, but was active against 3 of the strains in A-No.3 medium (MICs: 12.5~25 μg/ml). In mice infected with the above strains, nocardicin A was effective against infections due to the same 3 strains which were susceptible to the drug in A-No.3 medium. These results indicate a correlation between the *in vitro* activity in A-No.3 medium and *in vivo* activity in the case of *Ps.*

Table 4. Relation between *in vitro* activity of nocardicin A and protecting effect against infections in mice

Organism	Challenge dose cells/mouse	ED_{50} (mg/kg)	MIC (μg/ml), 10^6/ml	
			HI agar	A-No. 3 agar
Ps. aeruginosa 191	4.9×10^4 (MLD)	24.5	>800	25
Ps. aeruginosa 195	6.0×10^6 (MLD)	35.2	>800	25
Ps. aeruginosa 201	6.0×10^6 (MLD)	59.5	>800	12.5
Ps. aeruginosa 161	7.8×10^5 (MLD)	>250.0	>800	800
Pr. mirabilis 60	6.0×10^6 (MLD)	4.5	100	6.25*
Pr. mirabilis 100	1.0×10^6 (10 MLD)	5.2	200	12.5*
Pr. mirabilis 105	8.2×10^7 (MLD)	21.7	100	12.5*

* A-No. 5 agar

Table 5. Mean serum levels and urinary excretion of nocardicin A and carbenicillin in mice after subcutaneous administration

Dose (mg/kg)	Antibiotic	Mean serum levels±S.E. (μg/ml)			Serum half life (min.)	Mean urinary excretion (0~24 hr)	
		1/4 hour	1/2 hour	1 hour		μg/ml	% recovery
40	Nocardicin A	32.8±4.0	21.7±1.0	8.2±0.4	23	148±13*	47.9±4.1**
	Carbenicillin	28.2±3.7	15.3±1.3	7.2±0.6	18	236±25	56.7±2.5
80	Nocardicin A	84.6±7.9	41.6±2.1	24.4±3.4	23	N.D.***	N.D.
	Carbenicillin	61.6±3.9	31.2±3.4	11.5±1.1	19		

* Mean urinary levels ±S.E. ** Mean recovery %±S.E. *** not done

Table 6. Hepatic and renal levels of nocardicin A and carbenicillin in mice after subcutaneous administration

Tissue	Dose (mg/kg)	Antibiotic	Tissue levels (μg/g)				
			1/2 hour	1 hour	4 hours	6 hours	8 hours
Liver	40	Nocardicin A	258	297	162	81	42
		Carbenicillin	132	45	<19	<19	<19
	80	Nocardicin A	360	380	252	136	57
		Carbenicillin	405	153	<19	<19	<19
Kidneys	40	Nocardicin A	35	14	<7	<7	<7
		Carbenicillin	36	21	<19	<19	<19
	80	Nocardicin A	48	28	<7	<7	<7
		Carbenicillin	80	30	<19	<19	<19

aeruginosa. *Pr. mirabilis* strains were also highly resistant to nocardicin A in HI medium, whereas the MICs of the drug against these strains in A-No.5 medium were 6.25~12.5 μg/ml. A satisfactory therapeutic effect against these strains was obtained as shown by ED_{50} values of 4.5~21.7 mg/kg. That is, the therapeutic effect of nocardicin A against infections caused by *Pr. mirabilis* did not reflect the MICs of the drug against the same strains in HI medium, but did reflect the MICs of nocardicin A in A-No.5 medium.

5. Serum Levels, Tissue Levels and Urinary Excretion in Mice

Nocardicin A and carbenicillin were given subcutaneously in single doses of 40 mg/kg and 80 mg/kg to mice. Serum, hepatic and renal levels of nocardicin A were compared with those of carbenicillin. As shown in Table 5, the serum levels of nocardicin A were slightly higher than those of carbenicillin at the above doses. The serum half-life was 23 minutes for nocardicin A and 18~19 minutes for carbenicillin. The mean rate of recovery in the 24-hour urine was 47.9% for nocardicin A and 56.7% for carbenicillin. As shown in Table 6, hepatic concentrations of nocardicin A were higher and more prolonged than those of carbenicillin. Conversely, renal concentrations of both drugs were lower than hepatic concentrations and were not prolonged. The higher and longer-lasting concentrations of nocardicin A in the liver of mice are considered to be one of the basic factors contributing to its potent therapeutic effect.

Discussion

In our preliminary evaluation of nocardicin A, we encountered the fact that the drug exerts an excellent therapeutic effect against infections in mice in spite of its low *in vitro* activity against certain kinds of gram-negative organisms in conventional HI medium. To elucidate this phenomenon the relation between the *in vitro* and *in vivo* activities of nocardicin A was studied and reported in this paper. As reported in the previous paper[2], it has been clarified that the *in vitro* activity of nocardicin A against *Ps. aeruginosa* and *Pr. mirabilis* was decreased by the high amounts of sodium chloride present in HI medium, but the drug was considerably active against these organisms in A-No.3 medium and A-No.5 medium which are low in sodium chloride content. The therapeutic effect of the drug in mice infected with these organisms was reflected in the MICs obtained in A-No.3 medium and A-No.5 medium. On the other hand, *E. coli* and *Staphylococcus aureus* were moderately or highly resistant *in vitro* to nocardicin A in all the medium tested. Nocardicin A was effective against infections due to *E. coli*, but ineffective against those due to *S. aureus*. These results suggest that the therapeutic efficacy of nocardicin A cannot be properly estimated by its MIC alone. This may be explained by the following: (1) The therapeutic efficacy of nocardicin A against infections in mice may not be affected by the inhibitors existing in conventional HI medium. (2) The activity of nocardicin A against *Ps. aeruginosa*, *Pr. vulgaris* and *E. coli* increases in the presence of polymorpho-nuclear leukocytes. The former may be explained by the possibility that the concentrations of inhibitors in the living body are not high enough to suppress the activity of nocardicin A. The latter supposition may be explained by the fact that nocardicin A in the presence of PMN acts synergistically against *E. coli* but not against *S. aureus*, as reported in our last paper.[1]

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