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

ABSORPTION, EXCRETION AND TISSUE DISTRIBUTION IN ANIMALS

YASUHIRO MINE, SHIGEO NONOYAMA, HITOSHI KOJO, SHIGEMI FUKADA and MINORU NISHIDA

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan

SACHIKO GOTO and SHOGO KUWAHARA

Department of Microbiology, Toho University, School of Medicine, Tokyo, Japan

(Received for publication July 12, 1977)

The absorption, excretion and tissue distribution of nocardicin A, a new monocyclic β-lactam antibiotic, were studied in various animals. When nocardicin A was given intramuscularly in single doses of 20 mg/kg to rats, rabbits, and dogs, the peak serum levels of nocardicin A were about 1.6~2.8 times higher than those of carbenicillin in all animals though the levels varied among the species tested. The serum half-life of nocardicin A in these animals was about twice that of carbenicillin. The 24-hour urinary recovery rate of nocardicin A after intramuscular injection was 68.5% in rabbits and 77.0% in dogs, but was low in rats; *i.e.*, 0.7%. When nocardicin A was given intravenously in single doses of 20 mg/kg to these animals, the peak serum levels varied widely among the test species; *i.e.* about 3 times higher than those of carbenicillin in rabbits and dogs, similar to those in rats. The peak serum and tissue levels of nocardicin A after intramuscular to intravenous injection were the highest in the kidneys, followed by the liver, serum, lungs, heart and spleen. The levels in the liver were prolonged. Nocardicin A, and traces of unknown substances less active than nocardicin A were observed as active substances in the urine recovered after injection of nocardicin A.

The *in vitro* and *in vivo* activities of nocardicin A were reported in our previous papers[1~3]. This paper deals with the results of absorption and excretion studies of nocardicin A in various animals.

Materials and Methods

1. Antibiotics

Antibiotics tested were nocardicin A (Fujisawa Research Laboratories) and carbenicillin (CBPC, Beecham Research Laboratories).

2. Animals

Animals used were male SD strain rats aged 6 weeks, weighing 180~220 g, male albino rabbits, weighing 2.1~2.9 kg, male beagle dogs, weighing 7.5~14.0 kg.

3. Bioassay

Bioassay was conducted by the cylinder-plate method or the disc-plate method with *Alcaligenes faecalis* 773-9 for nocardicin A and *Pseudomonas aeruginosa* NCTC-10490 for carbenicillin as the test organisms.

4. Stability in tissue homogenates of animals

With M/15 phosphate buffer (pH 7.0) 33% homogenates were prepared from the kidneys, lungs, liver and spleen of rats, and the kidneys of mice and rabbits. One volume of 2,000 μg/ml nocardicin A solution was mixed with 9 volumes each of the above homogenates, and incubated for 30, 60 or 90 minutes at 37°C. An equal volume of 99% ethanol was added to the incubation mixture to terminate enzymatic reaction, and the supernatant fluid obtained by centrifugation was assayed by the disc-plate method.

5. Serum levels and urinary excretion

Nocardicin A or carbenicillin was given intramuscularly or intravenously to animals in single doses of 20 mg/kg. Blood specimens were removed periodically and the serum was separated. Urine was collected at regular intervals up to 24 hours after injection. Standard curves prepared with normal serum and M/15 phosphate buffer (pH 7.0) respectively were obtained to determine serum and urinary concentrations of nocardicin A.

6. Biliary excretion

Dogs were anesthetized and a polyethylene tube was inserted in the bile duct. The bile was collected periodically through the tube after intramuscular injection of 20 mg/kg of nocardicin A.

7. Tissue distribution

Nocardicin A was given intramuscularly or intravenously to rabbits in single doses of 100 mg/kg. Rabbits in groups of 3 each were bled to death at 0.5 and 2 hours after intramuscular injection, and at 0.25 and 1 hour after intravenous injection. The lungs, heart, liver, spleen and kidneys were removed. Tissues from each of the organs were homogenized in a weight of M/15 phosphate buffer (pH 7.0) twice that of the tissue. The antibiotic concentrations in the supernatant, obtained by centrifuging the homogenate at $10,000 \times g$ for 20 minutes, were bioassayed with the standard solution prepared with M/15 phosphate buffer (pH 7.0).

8. Active substance in urine

Nocardicin A was given intramuscularly to various animals in single doses of 20 mg/kg. The urine was collected over a period of 24 hours after injection. The urine was spotted on Eastman Chromatogram Sheet No. 6060 and chromatographed with a *n*-butanol solvent system (*n*-butanol - acetic acid - water, 4: 1: 2, v/v). Bioautography was then performed using *Alcaligenes faecalis* 773-9 and *Ps. aeruginosa* III (permeable mutant to *Ps. aeruginosa* NCTC-10490) as the test organisms.

Results

1. Serum Levels and Urinary Excretion after Intramuscular Injection

Table 1 shows the mean serum levels and the serum half-lives of nocardicin A and carbenicillin after single intramuscular doses of 20 mg/kg each of rats, rabbits and dogs (in groups of 5). The peak serum levels of nocardicin A were 24.3 μg/ml in rats, 48.2 μg/ml in rabbits, and 34.1 μg/ml in dogs. These peak values were 1.6 ~ 2.8 times higher than those of carbenicillin in all the animals tested. The serum half-life of nocardicin A was 61 ~ 78 minutes in rabbits and dogs; *i.e.*, 1.6 ~ 1.9 times longer than that of carbenicillin. On the other hand, the serum half-lives of nocardicin A and carbenicillin in rats were shorter than those in other animals, that is, 23 minutes and 13 minutes, respectively. Five dogs were given intramuscularly varying doses of nocardicin A and the serum levels were compared in a crossover study. As shown in Fig. 1, the peak serum levels at single doses of 20 mg/kg, 40 mg/kg and 80 mg/kg were 34.6 μg/ml, 62.0 μg/ml and 120.6 μg/ml respectively, indicating a strong dose-response relationship. The 24-hour urinary recovery rate of nocardicin A after a single intramuscular dose of 20 mg/kg was comparatively high in rabbits and dogs; 68.5% and 77.0% respectively, but was very low (0.7%) in rats (Table 2). The urinary excretion of nocardicin A was not uniform among the test species, and except in dogs, was generally lower than that of carbenicillin. This tendency was especially marked in rats.

2. Serum Levels and Urinary Excretion after Intravenous Injection

Table 3 shows the mean serum levels of nocardicin A and carbenicillin in various animals after a single intravenous injection of 20 mg/kg. The peak serum levels of nocardicin A and carbenicillin in rats were 23.3 μg/ml and 20.5 μg/ml respectively and the serum half-lives of both antibiotics were

Table 1. Mean serum levels of nocardicin A and carbenicillin in various animals after intramuscular injection (20 mg/kg)

Animal	Antibiotic	Mean serum levels, μg/ml, n=5						Half-life (min.)
		1/2 hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	
Rat	Nocardicin A	24.3±1.3*	10.0±0.8	<1.0	<1.0	<1.0	<1.0	23
	Carbenicillin	14.4±1.2	3.1±0.3	<1.0	<1.0	<1.0	<1.0	13
Rabbit	Nocardicin A	48.2±3.1	31.3±3.8	17.5±2.5	9.0±1.8		2.2±1.1	61
	Carbenicillin	17.5±1.2	10.1±0.8	3.8±0.7	<2.0	<2.0	<2.0	38
Dog	Nocardicin A	34.1±3.2	34.6±2.8	21.3±0.5	12.7±1.0		3.4±0.4	78
	Carbenicillin	22.8±0.9	16.7±0.2	6.8±0.4	2.3±0.3	<2.0	<2.0	42

* Mean serum levels ±S.E.

11~12 minutes. However, there was a marked difference between serum levels of nocardicin A and carbenicillin in rabbits and dogs. That is, in rabbits the peak serum level of nocardicin A was 100.4 μg/ml in comparison with 36.4 μg/ml of carbenicillin, and in dogs the peak serum levels of nocardicin A was 82.0 μg/ml in comparison with 27.7 μg/ml of carbenicillin. The mean serum half-life of nocardicin A in rabbits and dogs was 41~49 minutes.

To investigate the dose-response relationship of a single dose, 5 dogs were given intravenously different amounts of nocardicin A and the serum levels were compared in a crossover study (Fig. 2). At single doses of 20 mg/kg, 40 mg/kg and 80 mg/kg, the dose-response relationship was less evident than that seen with the same dose given intramuscularly. The urinary excretion of nocardicin A and carbenicillin in

Fig. 1. Mean serum levels of nocardicin A in dogs after single intramuscular injections of 20, 40 and 80 mg/kg.

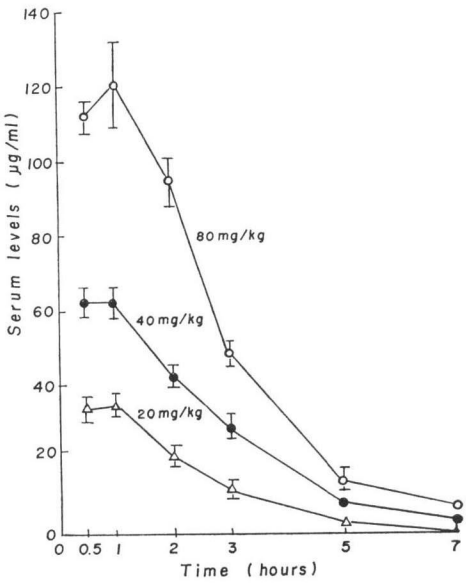


Table 2. Mean urinary excretion of nocardicin A and carbenicillin in various animals after intramuscular injection (20 mg/kg)

Animal	Antibiotic	0~3 hrs.		3~6 hrs.		6~24 hrs.		0~24 hrs %
		μg/ml	%*	μg/ml	%	μg/ml	%	
Rat	Nocardicin A	18±4.7**	0.6	3.8±1.1	0.1	—	—	0.7±0.04
	Carbenicillin	1188±178	50.6	52.8±14.7	1.0	—	—	52.0±1.0
Rabbit	Nocardicin A	1043±345	55.2	390.0±147	9.9	7.3±2.0	3.5	68.5±9.3
	Carbenicillin	1636±367	83.5	252.0±92	9.7	—	—	93.1±1.2
Dog	Nocardicin A	6490±1874	50.4	3036.0±701	16.1	267.0±79	10.5	77.0±2.6
	Carbenicillin	2635±232	46.7	951.0±184	7.7	6.9±4.7	0.1	54.6±3.0

* % recovery, ** Mean urinary level ±S.E.

Table 3. Mean serum levels of nocardicin A and carbenicillin in various animals after intravenous injection (20 mg/kg)

Animal	Antibiotic	Mean serum levels, μg/ml, n=5						Half-life (min.)
		1/4 hr.	1/2 hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	
Rat	Nocardicin A	23.3±1.2*	6.2±0.5	1.8±0.2	<0.9	<0.9	<0.9	11
	Carbenicillin	20.5±1.8	9.1±0.5	<2.0	<2.0	<2.0	<2.0	12
Rabbit	Nocardicin A	100.4±4.8	54.6±5.3	30.3±3.7	13.9±1.5	6.8±0.7		49
	Carbenicillin	36.4±1.2	16.5±1.3	2.9±0.3	<2.0	<2.0		12
Dog	Nocardicin A	82.0±3.4	57.0±2.4	36.0±1.6	12.8±1.0	6.2±0.7		41
	Carbenicillin	27.7±1.1	15.9±1.0	9.5±0.7	4.9±0.3	2.2±0.1		53

* Mean serum level ±S.E.

Table 4. Mean urinary excretion of nocardicin A and carbenicillin in various animals after intravenous injection (20 mg/kg)

Animal	Antibiotic	0~3 hrs.		3~6 hrs.		6~24 hrs.		0~24 hrs.
		μg/ml	%*	μg/ml	%	μg/ml	%	%
Rat	Nocardicin A	138±33.1**	6.0	<2.0	—	<2.0	—	6.0±1.2
	Carbenicillin	856±116	66.4	9.3±2.2	0.2	<4.0	—	66.6±1.3
Rabbit	Nocardicin A	1593±289	63.6	443.0±56	13.5	11.6±2.3	5.0	81.2±1.6
	Carbenicillin	1942±370	33.2	698.0±145	25.6	10.8±2.1	3.8	62.6±3.8
Dog	Nocardicin A	5620±1370	79.4	521.0±186	6.2	37.0±8.9	2.0	87.6±5.5
	Carbenicillin	2520±340	32.2	562.0±242	6.1	54.2±32.7	1.3	39.7±4.9

* % recovery, ** Mean urinary levels ± S.E.

Table 5. Mean biliary excretion of nocardicin A in dogs after intramuscular injection (20 mg/kg)

Animal	0~3 hrs.		3~6 hrs.		6~24 hrs.		0~24 hrs.
	μg/ml	%*	μg/ml	%	μg/ml	%	%
Dog	11.0±1.6**	0.05	11.1±1.7	0.02	9.7±1.4	0.05	0.12±0.03

* % recovery, ** Mean biliary level ± S.E.

various animals after intravenous injection of 20 mg/kg is shown in Table 4. As with the intramuscular injection, the urinary recovery rate of nocardicin A varied among species from 81.2~87.6% except in rats which was as low as 6.0%.

3. Biliary Excretion

Table 5 shows the biliary excretion of nocardicin A in dogs after an intramuscular injection of 20 mg/kg. The biliary excretion of this antibiotic was very low; the biliary levels were 9.7~11.1 μg/ml and the total recovery rate for 24 hours was 0.12%.

4. Tissue Distribution

Table 6 shows the tissue distribution of nocardicin A in rabbits after an intramuscular or intravenous injection of 100 mg/kg. The levels of nocardicin A both after intramuscular and intravenous injections were the highest in the kidneys, followed by the liver, lungs, heart and spleen. Hepatic con-

Table 6. Tissue distribution of nocardicin A in rabbits after intramuscular or intravenous injection (100 mg/kg)

Organ	Intramuscular injection		Intravenous injection	
	1/2 hr	2 hrs.	1/4 hr.	1 hr.
Liver*	73.0	156.0	176.0	252.0
Kidney*	268.0	250.0	845.0	371.0
Lung*	47.8	42.1	113.0	66.4
Heart*	41.3	28.6	89.0	47.5
Spleen*	19.5	22.5	52.8	29.3
Serum**	130.0	94.3	312.3	131.3

* μg/g, ** μg/ml

Table 7. Active substances recovered in urine of various animals after intramuscular injection of nocardicin A

Animal	Diameter (mm) of inhibition zone			
	Alcaligenes faecalis		*Ps. aeruginosa* III	
	Rf: 0.52	Rf: 0.88	Rf: 0.52	Rf: 0.88
Mouse	23.0	—	34.0	—
Rat	28.0	—	39.0	25.0
Rabbit	26.0	—	39.0	8.0
Dog	31.0	—	42.0	11.0

TLC; Solvent system (*n*-butanol - acetic acid - water, 4: 1: 2, v/v), Adsorbent (Eastman Chromatogram Sheet No. 6060)

Test organism; *Alcaligenes faecalis* 773-9, *Ps. aeruginosa* III (permeable mutant of *Ps. aeruginosa* NCTC-10490)

Rf value; Rf: 0.52=nocardicin A,
Rf: 0.88=unknown substance

Fig. 2. Mean serum levels of nocardicin A in dogs after single intravenous injections of 20, 40 and 80 mg/kg.

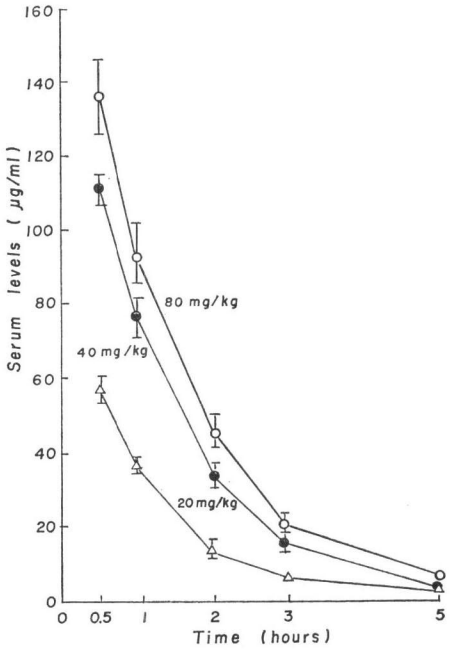
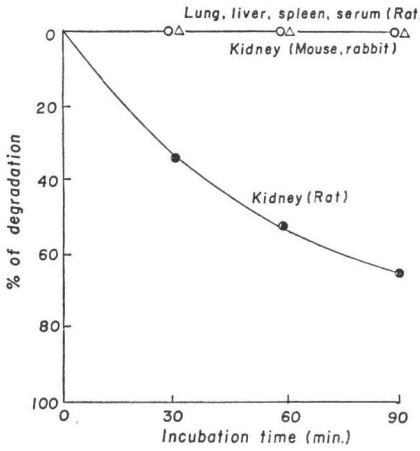


Fig. 3. Stability of nocardicin A in tissue homogenates.



centration of nocardicin A was higher 2 hours after intramuscular injection than at 30 minutes, but no great differences in concentrations in other tissues except heart were observed between 30 minutes and 2 hours. Moreover, the concentration of nocardicin A in the liver was prolonged after intravenous injection, but the tendency of prolongation in the other tissues was not so pronounced as after intramuscular injection.

5. Stability of Nocardicin A in Tissue Homogenates of Animals

Although nocardicin A was very stable in serum and liver, lungs and spleen homogenates of rats, it was rapidly decomposed in the kidney homogenate. However, the decomposition of nocardicin A in kidney homogenate was specific in the case of rat, and was not observed in mouse and rabbit (Fig. 3).

Table 8. Mean serum levels and urinary excretion of nocardicin A in rabbits after oral administration (100 mg/kg)

Animal	Mean serum levels, μg/ml, n=5					Urinary excretion (0~24 hours)
	1/2 hr.	1 hr	2 hrs.	3 hrs.	5 hrs.	
Rabbit	1.3±0.1*	2.2±0.4	2.8±0.3	2.7±0.3	1.5±0.2	2.7±1.3%**

* Mean serum level ± S.E., ** % recovery

6. Active Substances in the Urine

Table 7 gives the active substances in the urine of various animals receiving nocardicin A assayed by thin-layer chromatography and bioautography. Bioautographic examination of the urine showed nocardicin A and traces of an unknown substance less active than nocardicin A when *Ps. aeruginosa* III was used as the test organism. But when *Alcaligenes faecalis* 773-9 was used as the test organism, only nocardicin A was detected as an active substance. This unknown substance tended to be excreted in a large quantity in rats and the recovery rate of nocardicin A was lower than in rabbits and dogs.

7. Serum Levels and Urinary Excretion in Rabbits after Oral Administration

Table 8 presents the serum levels and urinary excretion of nocardicin A in rabbits after oral administration of 100 mg/kg. The peak serum level was as low as 2.8 μg/ml and the urinary recovery was only 2.7%. This shows that nocardicin A was poorly absorbed when given orally.

Discussion

As previously reported[1~5], nocardicin A is a new monocyclic β-lactam antibiotic with a unique antimicrobial activity. Its comparatively strong therapeutic effect in infected mice cannot be explained sufficiently from *in vitro* antimicrobial activity alone. However, the fact that the *in vitro* antimicrobial activity of nocardicin A against *Ps. aeruginosa*, *E. coli* and *Pr. vulgaris* was enhanced in the presence of fresh serum or polymorphonuclear leukocytes is considered to be one of the basis supporting the potent *in vivo* efficacy of the antibiotic. The levels of nocardicin A in the body of various animals were compared with those of carbenicillin. In all the test species, the serum levels of nocardicin A after intravenous administration were higher than those of carbenicillin. However, the serum levels varied widely among the species; the mean peak level (24.3 μg/ml) in rats after intravenous injection of 20 mg/kg was about half that (48.2 μg/ml) in rabbits. The serum half-life of nocardicin A in rats was short, *i.e.* less than half that in the 2 other species. The 24-hour urinary recovery of nocardicin A in rats after intramuscular injection was only 0.7%; very low even after taking into account the serum levels. This seems to be due to the fact that nocardicin A is inactivated rapidly *in vitro* in renal homogenates of rats and suggests that rats are not appropriate subjects for pharmacokinetic studies of nocardicin A. The 24-hour urinary excretion rate of nocardicin A after intravenous injection was very low (about 6.0%) in rats; the same as in the case with intramuscular injection, but was generally higher than after intramuscular injection in rabbits and dogs. It remains to be clarified which animals humans most closely resemble in absorption and excretion of nocardicin A and whether the excretion rate of the antibiotic in humans differs as in animals according to the route of administration.

Acknowledgement

The authors wish to thank Dr. H. NAKANO, Director of Research of Fujisawa Pharmaceutical Co., Ltd. and Dr. S. KUMADA, Director of Research Laboratories, for guidance and encouragement.

References

1) NISHIDA, M.; Y. MINE, S. NONOYAMA, H. KOJO, S. GOTO & S. KUWAHARA: Nocardicin A, a new monocyclic β-lactam antibiotic. III. *In vitro* evaluation. J. Antibiotics 30: 917~925, 1977
2) KOJO, H.; Y. MINE, M. NISHIDA & T. YOKOTA: Nocardicin A, a new monocyclic β-lactam antibiotic. IV. Factors influencing the *in vitro* activity of nocardicin A. J. Antibiotics 30: 926~931, 1977
3) MINE, Y.; S. NONOYAMA, H. KOJO, S. FUKADA, M. NISHIDA, S. GOTO & S. KUWAHARA: Nocardicin A, a new monocyclic β-lactam antibiotic. V. *In vivo* evaluation. J. Antibiotics 30: 932~937, 1977
4) AOKI, H.; H. SAKAI, M. KOHSAKA, T. KONOMI, J. HOSODA, Y. KUBOCHI, E. IGUCHI & H. IMANAKA: Nocardicin A, a new monocyclic β-lactam antibiotic. I. Discovery, isolation and characterization. J. Antibiotics 29: 492~500, 1976
5) HASHIMOTO, M.; T. KOMORI & T. KAMIYA: Nocardicin A, a new monocyclic β-lactam antibiotic. II. Structure determination of nocardicins A and B. J. Antibiotics 29: 890~901, 1976