Production of Nocardicyclins by Clinical Isolates of *Nocardia pseudobrasiliensis* and *In Vivo* Antitumor Activity of the Antibiotic

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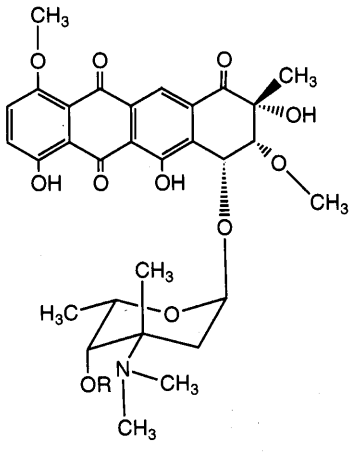
We previously isolated and reported new bioactive metabolites[1~4] from clinical isolates of pathogenic *Nocardia* and our continuing studies on the production of secondary metabolites by pathogenic *Nocardia* showed that these are limited to *Nocardia brasiliensis* strains[5]. Isolates of *N. brasiliensis* have not been considered taxonomically heterogenous in comparison with those of *N. asteroides* and *N. farcinica*, although most studies have not included large numbers of isolates[6]. Recently, however, RUIMY *et al.*[7] proposed a new taxon among isolates of *N. brasiliensis* associated with invasive nocardiosis. This new taxon, *N. pseudobrasiliensis* differs from *N. brasiliensis* in the hydrolysis of adenine, β-lactamase patterns on isoelectric focusing, and specific mycolic acid pattern[6], but clinical recognition is difficult because these tests are not routinely used in the clinical laboratory. Simple tests such as antibiotic production for the identification of this species are still required.

We recently isolated and reported the new anthracycline antibiotics, nocardicyclins A and B[3] (Fig. 1) from a type strain of *N. pseudobrasiliensis* IFM 0624^T. We could not confirm the production of the antibiotics from *N. brasiliensis* sensu stricto when we tested 84 strains of *N. brasiliensis*[5], and therefore, we were interested if this is a species-specific character of *N. pseudobrasiliensis*. We therefore examined the production of nocardicyclins by other clinical isolates of *N. pseudobrasiliensis*. In the current paper we also report on the *in vivo* antitumor activity of nocardicyclin A against P388 and P388/ADR leukemias in mice.

Six strains of *N. pseudobrasiliensis* (IFM 0756, 0757, 0758, 0759, 0760, 0761), which were isolated from patients with invasive pulmonary nocardiosis, were gifts from Department of Microbiology University of Texas Health Center at Tyler, USA. A loopful of the slant culture of each strain was inoculated into a 500-ml Erlenmeyer flask containing 100 ml of a medium consisting of 2% glucose brain heart infusion broth (BHI medium, Difco). The seed culture (5%) was transferred to 20 liters of production medium (pH 7.0 before sterilization) contained of glucose 1.0%, glycerol 1.0%, polypepton 1.0%, Adekanol (Asahi Denka Co., Ltd.) 0.1% and meat extract 0.5%. The fermentation was carried out at 32°C for 4 days under aeration of 15 liters/minute and agitation at 200 rpm. Production of nocardicyclins A and B were monitored by HPLC (Lichrospher RP-18e, 4.6 × 150 mm, Merck) using 22% CH_3CN with 0.2% TFA as a mobile phase. Retention times of standard nocardicyclins A (7.22 minutes) and B (13.84 minutes) under this HPLC condition were used for the comparative studies. Our HPLC studies indicated that the strains IFM 0757, 0758 and 0760 produce nocardicyclins, but the production of these compounds by strains IFM 0755, 0759 and 0761 were not observed. In this study, we could also confirm our previous findings that *N. pseuodobrasiliensis* IFM 0624 (JCM 9894) is a producer of the antibiotics, in addition to the type strain IFM 0624^T (JCM 9894)[3]. All these studies indicated that of 8 strains of *N. pseudobrasiliensis* (including the type strain IFM 0624^T) tested, 5 strains were producers of both antibiotics. In the remaining 3 strains of *N. pseudobrasiliensis*, production of nocardicyclins was not confirmed, but interestingly, weak antibacterial activity was detected. The amount of the active principle(s) produced, however, was not enough to

Fig. 1. Structures of nocardicyclins A and B.



Nocardicyclin A: R = H
Nocardicyclin B: R = $COCH_3$

Table 1. *In vivo* antitumor activity of nocardicyclin A.

Implanted cell line	Drug	Dose[a] (mg/kg)	MST (SD)[b] (days)	T/C (%)	Av. body weight on day 10 (g)
P388	Control	—	10.4 (0.97)	100.0	24.8
	Nocardicyclin A	1	12.2 (2.12)*	118.0	26.5
		5	13.4 (1.33)*	129.1	25.1
		10	15.0 (0)*	144.8	24.4
	Doxorubicin	1	12.4 (4.58)*	119.9	23.2
P388/ADR	Control	—	11.1 (0.32)	100.0	26.8
	Nocardicyclin A	1	13.8 (2.88)*	124.8	25.6
		5	14.3 (3.53)*	129.2	27.2
		10	16.5 (4.35)*	148.7	27.4
	Doxorubicin	1	10.7 (1.75)	96.1	23.5

[a] Each drug administered intrapertioneally from days 0 to 9.
[b] Mean survival time (standard deviation).
* $p<0.01$ against control by Mann-Whiteney's U-test.

characterize the physico-chemical properties of the antibacterial principle. Detailed chemical studies of these secondary metabolites are necessary because the nature of these compounds may be useful as chemotaxonomic characters for the identification of *Nocardia pseudobrasiliensis*. Further studies using many strains of *N. pseudobrasiliensis* are also in progress to determine wether the production of nocardicyclins is a stable species-diagnostic character.

The antitumor activity of nocardicyclin A against P388 leukemia and its multi-drug resistant cell line of P388/ADR leukemia is shown in Table 1. Both leukemia cells were implanted intraperitoneally (ip) at 5×10^5 cells/mouse into CDF_1 aged 6 weeks (weighing about 17 g). The treatment started 3 hours after the tumor implantation and the drugs were given ip from day 0 to day 9. Nocardicyclin A showed a significant prolongation of mean survival time (MST) against P388 leukemia at doses of 1, 5 and 10 mg/kg for 10 consecutive days, and their T/C values obtained were 118.0, 129.1 and 144.8%, respectively. Nocardicyclin A also exhibited a similar T/C values against multi-drug resistance P388/ADR leukemia implanted mice, and the T/C values obtained at each dosis were 124.8, 129.2 and148.7%, respectively. Doxorubicin, on the other hand, did not show any antitumor activity against P388/ADR leukemia in mice. We had reported that cytotoxic activity of nocardicyclin A and doxorubicin against P388 and P388/ADR were 0.47 and 0.97, and 0.09 and 0.45 mg/ml, respectively, indicating that nocardicyclin A was less toxic than doxorubicin. This low toxicity of nocardicyclin A was further confirmed by the *in vivo* experiments that the LD_{50} value of nocardicyclin A for ICR mice with single ip injection was >100 mg/kg/mouse.

The reason why nocardicyclin A exhibited an equivocal antitumor activity against P338/ADR as well as P388 leukemia is not clear. Since it is reasonable to consider that nocardicyclin A has the same mechanism as doxorubicin, the higher activity of nocardicyclin A against P388/ADR may be due to a characteristic pharmacodynamic behavior of the antibiotic. Therefore, coupled with its lower toxicity, nocardicyclin A merits further evaluation as a cancer chemotherapeutic.

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