Differentiation between the Genera *Rhodococcus* and *Nocardia* and between Species of the Genus *Mycobacterium* by Susceptibility to Bleomycin

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Susceptibility to bleomycin proved to be a useful criterion for differentiating *Rhodococcus* from *Nocardia*, the former being susceptible and the latter resistant to this agent. The test was also useful for discriminating between various species of the genus *Mycobacterium*.

INTRODUCTION

Recently susceptibility tests to 5-fluorouracil and to mitomycin C were reported to be useful for discriminating between the genera *Rhodococcus* and *Nocardia*, as the former was susceptible and the latter resistant to these agents (Tsukamura, 1981*a*, 1981*b*). Another anticancer agent, bleomycin, has been tested for its ability to differentiate between these two genera and between various species of the genus *Mycobacterium*.

METHODS

Strains. All strains used were from the collection of this laboratory.

Media. Modified Sauton agar and Ogawa egg media were used. The composition of the modified Sauton agar medium was as follows: glycerol, 30 ml; KH_2PO_4 , 0.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; citric acid, 2.0 g; ferric ammonium citrate, 0.05 g; sodium glutamate, 4.0 g; purified agar (Eiken Company, Tokyo), 20.0 g; distilled water, 970 ml. The medium was adjusted to pH 7.0 with 10% (w/v) KOH solution. The medium was sterilized by autoclaving at 120 °C for 20 min. Bleomycin hydrochloride (Nippon Kayaku Company, Tokyo) was added aseptically to the medium after the latter had been liquefied by heating in a water-bath (100 °C). The following concentrations of bleomycin were used: 10, 5, 2.5, 1.25, and 0 $\mu\text{g ml}^{-1}$. The bleomycin-containing media were distributed in 7 ml quantities into tubes, 165 × 16.5 mm, and solidified as slopes. The composition of the Ogawa egg medium was as follows: basal solution [1% (w/v) KH_2PO_4 and 1% (w/v) sodium glutamate], 100 ml; whole eggs, 200 ml; glycerol, 6 ml; 2% (w/v) aqueous solution of malachite green, 6 ml (pH 6.8). Bleomycin and other anticancer agents (see Table 1) were added to the Ogawa egg medium before sterilization. The medium was distributed in 7 ml quantities into tubes, 165 × 16.5 mm, and sterilized by heating at 90 °C for 60 min, thereby coagulating it as slopes.

Test strains of rapidly growing mycobacteria, rhodococci and nocardiae were cultivated at 37 °C (nocardiae, 28 °C) for 5 d and slowly growing mycobacteria were cultivated at 37 °C for 14 d on Ogawa egg medium. From these cultures, a bacterial suspension, approximately 1 mg wet weight ml^{-1} , was prepared. A sample (0.02 ml) of these suspensions was inoculated on to each medium by use of a spiral loop. The medium was stoppered with a double-edged gum cap having a 3 mm cut in the centre of its base and incubated at 37 °C (nocardiae, 28 °C) for 5 d (slowly growing mycobacteria, 14 d). Confluent growth similar to that on the control medium was scored positive. Partial growth was observed sometimes, but this was regarded as negative. In most cases, the reading was clear-cut.

RESULTS AND DISCUSSION

The inhibitory activity of seven anticancer agents tested in Ogawa egg medium is shown in Table 1.

A pilot study on the action of bleomycin showed that the reading of the inhibitory concentration is more clear-cut with Sauton agar medium than with Ogawa egg medium. Hence, the test for susceptibility to bleomycin of rapidly growing mycobacteria, rhodococci, and nocardiae, which grow well on this medium, was carried out with modified Sauton agar medium. Slowly

Table 1. Comparison of susceptibility to various anticancer agents of *Mycobacterium*, *Rhodococcus* and *Nocardia* grown on Ogawa egg medium

Anticancer agent*	Minimum inhibitory concentration ($\mu\text{g ml}^{-1}$)			
	<i>Mycobacterium thermoresistibile</i>	<i>Mycobacterium aurum</i>	<i>Rhodococcus bronchialis</i>	<i>Nocardia asteroides</i>
	ATCC 19527	ATCC 23366	ATCC 25592	ATCC 19247
5-Fluorouracil	<5	<5	<5	>40
Mitomycin C	1.25	1.25	5	>10
Bleomycin hydrochloride	2.5	2.5	5	>20
Chromomycin A3	10	5	10	10
Doxorubicin hydrochloride	>20	>20	>20	>20
Cyclophosphamide	>20	>20	>20	>20
Cytosine arabinoside	>20	>20	>20	>20

* Sources: 5-Fluorouracil and doxorubicin hydrochloride, Kyowa Hakko Co., Tokyo; mitomycin C, Sanko Co., Tokyo; bleomycin hydrochloride, Nippon Kayaku Co., Tokyo; chromomycin A3, Takeda Chemical Co., Osaka; cyclophosphamide, Shionogi Co., Osaka; cytosine arabinoside, Nippon Shiyaku Co., Kyoto.

Table 2. Susceptibility to bleomycin of rapidly growing mycobacteria, rhodococci and nocardiae grown on modified Sauton agar medium

Figures in parentheses indicate numbers of strains that showed only thin confluent growth.

Species	No. of strains tested	No. of strains showing growth				
		Concn of bleomycin ($\mu\text{g ml}^{-1}$)				
		10	5	2.5	1.25	0
<i>Mycobacterium</i>						
Rapidly-growing, non-photochromogenic						
<i>M. smegmatis</i>	5	5	5	5	5	5
<i>M. fortuitum</i>	10	10	10	10	10	10
<i>M. chelonae</i> subsp. <i>chelonae</i>	10	10	10	10	10	10
<i>M. chelonae</i> subsp. <i>abscessus</i> *	10	4	4	8	10	10
<i>M. chitae</i>	5	0	0	3	5	5
<i>M. agri</i>	5	0	0	0	0	5
' <i>M. diernhoferi</i> '	5	0	0	0	0	5
Rapidly-growing, photochromogenic						
<i>M. vaccae</i>	5	0	0	1	2	5
<i>M. parafortuitum</i>	5	0	0	2	4	5
Rapidly-growing, scotochromogenic						
<i>M. phlei</i>	5	0	0	0	1	5
<i>M. flavescens</i>	5	0	0	0	0	5
<i>M. thermoresistibile</i>	5	0	0	0	5	5
<i>M. aurum</i> †	5	2	2	2	4	5
<i>M. rhodesiae</i>	5	0	0	4	5	5
<i>M. neoaurum</i>	5	5	5	5	5	5
<i>M. obuense</i>	5	(5)	(5)	5	5	5
<i>M. aichiense</i>	5	(5)	(5)	5	5	5
<i>M. chubuense</i>	5	0	(4)	5	5	5
<i>M. tokaiense</i>	4	0	0	2	4	4
' <i>M. austroafricanum</i> '	5	0	0	0	0	5
<i>Rhodococcus</i>						
<i>R. rhodochrous</i>	10	0	0	2	3	10
<i>R. bronchialis</i>	10	0	0	0	4	10
<i>R. rubropertinctus</i>	10	0	0	0	0	10
<i>R. terrae</i>	10	0	0	0	0	10
<i>R. ruber</i>	6	0	0	0	0	6
<i>R. equi</i>	2	0	0	0	0	2
<i>R. coprophilus</i>	2	0	0	0	0	2

Table 2 (continued)

Species	No. of strains tested	No. of strains showing growth				
		Concn of bleomycin ($\mu\text{g ml}^{-1}$)				
		10	5	2.5	1.25	0
<i>Nocardia</i>						
<i>N. asteroides</i>	33	22	25	28	29	33
<i>N. farcinica</i>	51	40	45	51	51	51
<i>N. brasiliensis</i>	5	4	5	5	5	5
<i>N. otitidis-caviarum</i>	9	9	9	9	9	9
' <i>Gordona</i> '						
' <i>Gordona aurantiaca</i> '	10	0	0	8	10	10

* Six strains including the type strain ATCC 19977 were susceptible to $5 \mu\text{g ml}^{-1}$ and four strains including the strain ATCC 14472 were resistant to $10 \mu\text{g ml}^{-1}$.

† The type strain ATCC 23366 was susceptible to $2.5 \mu\text{g ml}^{-1}$.

Table 3. Susceptibility to bleomycin of slowly growing mycobacteria grown on Ogawa egg medium

Species	No. of strains tested	No. of strains showing growth				
		Concn of bleomycin ($\mu\text{g ml}^{-1}$)				
		20	10	5	2.5	0
<i>M. tuberculosis</i>	5	0	0	0	0	5
<i>M. bovis</i>	5	0	0	0	0	5
<i>M. kansasii</i>	5	0	0	0	0	5
<i>M. marinum</i>	5	5	5	5	5	5
<i>M. simiae</i>	5	5	5	5	5	5
<i>M. asiaticum</i>	5	4	5	5	5	5
<i>M. avium</i>	20	14	16	20	20	20
<i>M. intracellulare</i>	30	5	7	13	27	30
<i>M. scrofulaceum</i>	10	0	0	0	0	10
<i>M. szulgai</i>	5	0	0	5	5	5
<i>M. gordonae</i>	10	1	1	5	8	10
<i>M. xenopi</i>	5	0	0	0	0	5
<i>M. shimoidei</i>	3	0	0	0	1	3
<i>M. nonchromogenicum</i>	10	8	8	10	10	10
<i>M. terrae</i>	10	4	4	4	6	10
<i>M. triviale</i>	10	0	0	0	0	10
<i>M. gastri</i>	5	0	0	0	0	5

growing mycobacteria were tested on Ogawa egg medium, as some species of this group showed only scanty or no growth on Sauton agar medium.

Apart from several strains of *Nocardia asteroides sensu stricto* (Tsukamura, 1969), all *Nocardia* strains were resistant to at least $2.5 \mu\text{g ml}^{-1}$ bleomycin, whereas almost all *Rhodococcus* strains were susceptible to this concentration (Table 2).

The test for susceptibility to bleomycin was also useful for differentiating between species of rapidly growing mycobacteria (Table 2). Of the rapidly growing, non-photochromogenic mycobacteria, only *M. smegmatis*, *M. fortuitum* and *M. chelonae* subsp. *chelonae* were resistant to $10 \mu\text{g ml}^{-1}$. Strains of *M. chelonae* subsp. *abscessus* were heterogeneous in respect to the susceptibility to bleomycin.

Of the slowly growing mycobacteria, *M. avium*, *M. intracellulare*, *M. scrofulaceum* and *M. asiaticum* were previously shown to be closely related to each other (Tsukamura, 1976). Of these four species, only *M. scrofulaceum* was sensitive to bleomycin (Table 3). *Mycobacterium nonchromogenicum*, *M. terrae* and *M. triviale* were also shown to be closely related to each other (Tsukamura, 1976), but only *M. nonchromogenicum* was resistant to bleomycin while *M. triviale* was sensitive. Strains of *M. terrae* showed varying degrees of susceptibility to bleomycin (Table 3). The type strain (ATCC 15755) of this species was resistant to $20 \mu\text{g ml}^{-1}$ bleomycin. *Mycobacterium gordonae* also was heterogeneous in its susceptibility to this agent.

REFERENCES

- TSUKAMURA, M. (1969). Numerical taxonomy of the genus *Nocardia*. *Journal of General Microbiology* **56**, 265–287.
- TSUKAMURA, M. (1976). Numerical classification of slowly growing mycobacteria. *International Journal of Systematic Bacteriology* **26**, 409–420.
- TSUKAMURA, M. (1981*a*). Differentiation between the genera *Mycobacterium*, *Rhodococcus* and *Nocardia* by susceptibility to 5-fluorouracil. *Journal of General Microbiology* **125**, 205–208.
- TSUKAMURA, M. (1981*b*). Test for susceptibility to mitomycin C as aids for differentiating the genus *Rhodococcus* from the genus *Nocardia*, and for differentiating *Mycobacterium fortuitum* and *Mycobacterium chelonae* from other rapidly growing mycobacteria. *Microbiology and Immunology* **25**, 1197–1199.