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Effect of Cortisone Administration on Experimental Nocardiosis

S. K. MISHRA, R. S. SANDHU, H. S. RANDHAWA, V. N. DAMODARAN, AND S. ABRAHAM

Departments of Medical Mycology and Pathology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi-7. India

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Effect of cortisone administration on the pathogenicity of *Nocardia asteroides*, *N. caviae*, and *N. brasiliensis* for white mice has been investigated by using the intravenous route of inoculation. The observations indicated that the susceptibility of white mice to nocardiosis was enhanced by cortisone. Test strains of the three species of *Nocardia* caused a higher and more rapid mortality, as well as more extensive lesions, in the cortisone-treated than in the normal mice. The mean lethal dose (LD_{50}) values of *N. asteroides* and *N. caviae* for the cortisone-treated group were found to be seven and eight times lower than their respective values for the normal group. *N. asteroides* and *N. caviae* were more virulent than *N. brasiliensis*, the LD_{50} of *N. brasiliensis* for cortisone-treated mice being 30 and 26 times higher than that of the former two species, respectively. *N. brasiliensis* also differed from the other two species in its inability to infect the brain. In the untreated animals, *N. asteroides* and *N. caviae* showed a tendency to form conglomerate growth, in contrast to formation of freely dispersed growth in the lesions of cortisone-treated animals.

During the past two decades, a number of investigators have attempted to study the effect of corticosteroids on the pathogenesis of experimental microbial infections in laboratory animals (1, 2, 4, 6-8, 10, 12-14 16). However, there is no information so far on the effect of corticosteroids on morbidity and mortality in experimental nocardiosis, although a number of reports have appeared concerning the pathogenicity of *Nocardia* species for normal laboratory animals and the chorioallantoic membrane of the developing chicken embryo (see reference 5). Results are presented of an investigation on the effect of cortisone administration on experimental nocardiosis in white mice caused by *N. asteroides*, *N. caviae*, and *N. brasiliensis*.

MATERIALS AND METHODS

Three strains of *N. asteroides* and two each of *N. caviae* and *N. brasiliensis* were used. The sources of test strains are given in Table 1. Six- to eight-week-old albino male mice bred in the animal house of Vallabhbhai Patel Chest Institute and each weighing about 20 g were used in this study. The test strains were grown for 10 to 15 days at 37 C in 100 ml of Dubos broth dispensed in 500-ml Erlenmeyer flasks. When sufficient growth had occurred, sterile glass beads of 3- to 5-mm diameter were introduced into the culture flasks which were agitated on a rotary shaker for 10 min. The suspension was allowed to stand for 5 min, and its upper portion was removed and centrifuged for 10 min at 3,000 rev/min. The sediment was homogenized in a sterile glass homogenizer; the homogenate was suspended in normal saline and filtered through steril cotton to remove the clumps. The filtrate was centrifuged, and the sediment was washed twice with sterile normal saline before resuspending in normal saline. A portion of this suspension was centrifuged in a Hopkin tube at 3,000 rev/min for 30 min, and the packed cell volume was recorded in microliters from the deposit in the graduated tip of the tube. One microliter was taken as equal to one milligram (wet weight) of the organisms (15). The suspension was adjusted so as to obtain the desired wet weight of the organisms per 0.2 ml of the suspension; this suspension was then injected in the tail vein of each animal. After an observation period of 3 or 6 weeks, the surviving animals were sacrificed. Cultures were made from brain, heart, lungs, liver, spleen, and kidneys of each animal, and portions of the same organs were fixed in 10% formol saline for histophatological examination. The tissue sections were stained with hematoxylin and eosin and Brown and Brenn modified Gram stain.

Administration of cortisone (Roussel, 25 mg of cortisone acetate per ml of suspension with 0.2% chloramphenicol as preservative) in the experimental animals was started 6 days prior to inoculation with

TABLE 1. *Sources of various strains of Nocardia species used in pathogenicity experiments*

Species	Accession no.	Source
Nocardia asteroides	Sp 27	Isolated from sputum of a case of pulmonary nocardiosis
N. asteroides	Lg 110	Isolated from cervical gland biopsy of a case of pulmonary nocardiosis
N. asteroides	Sl 1166	Soil isolate from Varanasi, Uttar Pradesh, India
N. caviae	Sl 163	Soil isolate from Kerala, India
N. caviae	IMRU 1259	Supplied by Ruth E. Gordon, Institute of Microbiology, Rutgers University, New Brunswick
N. brasiliensis	Sl 54	Soil isolate from Madhya Pradesh, India
N. brasiliensis	IMRU 1188	Supplied by Ruth E. Gordon

Nocardia. Each animal received, unless otherwise indicated, a total of 7.5 mg of the steroid in three equal doses injected intramuscularly in the hind legs on alternate days. In an exploratory experiment, this dosage of cortisone was found to be well tolerated by white mice and at the same time sufficient to enhance their susceptibility to infection when challenged by about 500 μg of inocula of each of the three test species of *Nocardia*.

RESULTS

Effect of cortisone on LD_{50}. For the determination of mean lethal dose (LD_{50}), inocula were prepared in fivefold dilutions from one strain each of *N. asteroides* (Sp 27), *N. caviae* (Sl 163), and *N. brasiliensis* (Sl 54). Six standard doses, i.e., 6,250, 1,250, 250, 50, 10, and 2 μg (wet weight) of the pathogen in 0.2 ml of saline suspension, were prepared. Groups of 10 normal (untreated) and 10 cortisone-treated mice, each animal receiving 7.5 mg of the steroid, were inoculated intravenously with each of the selected doses of inoculum. Thus, groups of 60 normal and 60 cortisone-treated mice were inoculated separately with *N. asteroides* and *N. caviae*. For *N. brasiliensis*, only 40 normal and 40 cortisone-treated mice were used because the two lowest doses of inocula were considered unsuitable for this species of relatively low virulence. In addition, 10 normal and 10 cortisone-treated mice were kept as controls for each of the test species. After 3 weeks of observation, the surviving animals were sacrificed, and the presence of gross and microscopic lesions on the organs was recorded. The data on mortality and morbidity among normal and cortisone-treated mice are presented in Table 2. The LD_{50} for the three species was computed according to the method of Reed and Muench (11). The maximum mortalities in normal mice obtained with various doses of *N. brasiliensis* did not exceed 20%, and hence it was not possible to determine the LD_{50} of this species. For *N. asteroides* and *N. caviae*, the LD_{50} values were 651 and 893 μg (wet weight) of cultures, respectively. For the cortisone-treated mice, LD_{50} values of *N. asteroides* and *N. caviae* were found to be about seven- and eightfold lower (95 and 110 μg, respectively) than those for the normal animals. The enhanced mortality in the cortisone group made it possible to determine the LD_{50} of *N. brasiliensis* which was 2,840 μg (wet weight) of cells. This value was nearly 30 and 26 times higher than that for *N. asteroides* and *N. caviae*, respectively. Mortalities due to *N. brasiliensis* appeared late, in contrast to the early mortalities caused by *N. asteroides* and *N. caviae* (Tables 2 and 3). The overall mortality due to the three species was comparatively more rapid in the cortisone-treated than in the normal mice. A majority of the animals challenged with *N. asteroides* and *N. caviae* showed gross and histopathological lesions on their lungs, kidneys, heart, and brain. Animals inoculated with *N. brasiliensis* also revealed active lesions on various organs, but the brain was never involved. All the infected organs yielded the pathogen in culture.

Pathogenicity for normal and cortisone-treated mice. For a detailed comparative study of the pathogenicity of *Nocardia* species, normal and cortisone-treated mice were inoculated intravenously with LD_{50} inocula as above. In all, three strains of *N. asteroides* and two strains each of *N. caviae* and *N. brasiliensis* were tested, and the data on mortality and morbidity for each strain during an observation period of 6 weeks are presented in Table 3. All the test strains of *N. asteroides* and *N. caviae* caused maximum mortality during the first 2 weeks. In the case of *N. brasiliensis*, the mortalities were delayed and occurred from the second week onward. There were no significant differences in the virulence of different strains of each of the three species. Many of the surviving animals in the normal and cortisone-treated groups showed active lesions due to the three species of *Nocardia* tested. The lesions in the treated animals were invariably more extensive than in the normal mice (Fig. 1-6), and histopathologically the following differences were noticeable in the two groups. (i) An acute inflammatory reaction was more marked in the lesions seen in the normal than in the cortisone-treated animals.

TABLE 2. *Data on mortality and morbidity among normal and cortisone-treated mice after intravenous challenge of graded inoculum of Nocardia asteroides, N. caviae, and N. brasiliensis*

Species (strain)	Inoculum (μg, wet weight)	Normal group[a]						Cortisone group[a]					
		No. of mice inoculated	Mortality				No. showing active lesions	No. of mice inoculated	Mortality				No. showing active lesions
			0-7 Days	8-14 Days	15-21 Days	Total			0-7 Days	8-14 Days	15-21 Days	Total	
N. asteroides (Sp 27)	2	10	0	0	0	0	0	10	0	0	0	0	0
	10	10	0	0	0	0	0	10	1	0	0	1	1
	50	10	0	1	0	1	0	10	3	0	1	4	5
	250	10	2	1	0	3	4	10	2	5	2	9	7
	1,250	10	3	4	0	7	6	10	10	0	0	10	8
	6,250	10	8	2	0	10	10	10	10	0	0	10	10
Total		60	13	8	0	21	20	60	26	5	3	34	31
N. caviae (Sl 163)	2	10	0	0	0	0	0	10	0	0	0	0	0
	10	10	0	0	0	0	0	10	1	1	0	2	1
	50	10	0	0	0	0	0	10	2	1	0	3	2
	250	10	0	1	0	1	3	10	6	1	1	8	6
	1,250	10	4	2	0	6	6	10	8	2	0	10	7
	6,250	10	5	5	0	10	8	10	10	0	0	10	9
Total		60	9	8	0	17	17	60	27	5	1	33	25
N. brasiliensis (Sl 54)	50	10	0	0	0	0	0	10	0	0	0	0	0
	250	10	0	0	0	0	0	10	1	1	0	2	2
	1,250	10	0	0	0	0	2	10	1	0	2	3	4
	6,250	10	0	1	1	2	3	10	1	3	3	7	8
Total		40	0	1	1	2	5	40	3	4	5	12	14

[a] For each group there were 10 mice constituting the control, and only one mortality was recorded in the cortisone-treated control animals.

(ii) The infecting strains of *Nocardia* species, more particularly *N. asteroides* and *N. caviae*, exhibited a tendency to form colonies (granule-like growth) in the organs of normal animals, whereas in the cortisone-treated group the organisms were more freely dispersed in the lesions (Fig. 7-12). (iii) Fatty liver changes following nocardial infection were observed in most of the cortisone-treated animals but not in the normal animals.

Further observations on the nature and extent of lesions produced are presented below for the individual species of *Nocardia*.

Nocardia asteroides. Five days after inoculation, the lesions were of pin-head size and usually marked by pus formation. They were more prominent in the lungs, measuring 2 to 5 mm in diameter (Fig. 1 and 2). Histopathologically, the lungs generally revealed multiple areas of abscess formation with hardly any intervening normal parenchyma. Lesions on the other organs were also in the form of abscesses, with typical organisms surrounded by inflammatory cells, mostly polymorphs and lymphocytes. The organisms usually occurred as gram-positive cocco-bacillary bodies or filaments with or without branching. Most of the animals sacrificed at the end of the 6-week observation period revealed healed lesions in which there were focal collections of fibroblasts and macrophages, but the organisms were no longer detectable. In some of the animals belonging to normal (untreated) as well as the cortisone-treated group, lesions were, however, still active.

Nocardia caviae. Macroscopic lesions due to *N. caviae* usually appeared, after a week, in the form of multiple circumscribed abscesses, measuring 1 to 2 mm in diameter (Fig. 3 and 4). Histopathologically, these abscesses showed nocardial granules as well as freely dispersed cocco-bacillary bodies and branching filaments which were accompanied by extensive pneumonic consolidations in the lungs. Surrounding the granules were inflammatory cells, chiefly comprising polymorphs and lymphocytes.

Nocardia brasiliensis. Both the test strains of *N. brasiliensis* caused only low mortalities among the normal mice even though the inocula were nearly nine and seven times higher than those used for *N. asteroides* and *N. caviae*, respectively. The gross lesions were usually noticeable 10 days after inoculation and were confined to the abdominal and thoracic organs. The abscesses were mostly cystic in appear-

TABLE 3. *Pathogenicity of different strains of Nocardia asteroides, N. caviae, and N. brasiliensis for normal and cortisone-treated mice*[a]

Species (strain)	Cortisone administered[b]	Inoculum (μg, wet weight)	No. inoculated	Mortality						No. showing gross lesions	No. showing histopathological lesions in:					
				1-7 Days	8-14 Days	15-21 Days	22-28 Days	29-42 days	Total		Brain	Heart	Lungs	Liver	Spleen	Kidneys
N. asteroides																
Lg 110	−	650	10	4	2	1	1	0	8	7	6	4	6	1	1	7
	+	100	10	5	2	1	1	0	9	9	5	6	8	4	1	7
Sp 27	−	650	10	3	2	1	0	0	6	8	6	3	6	2	0	8
	+	100	10	4	2	1	0	0	7	10	4	3	9	2	0	8
Sl 1166	−	650	10	4	0	0	0	0	4	5	4	3	3	2	0	5
	+	100	10	4	1	0	0	0	5	5	3	4	4	1	0	5
N. caviae																
Sl 163	−	900	10	2	2	1	1	0	6	7	3	3	5	1	0	6
	+	110	10	2	2	1	2	0	7	8	2	2	6	0	0	8
IMRU 1259	−	900	10	5	3	1	1	0	10	10	7	5	10	1	2	10
	+	110	10	6	2	2	0	0	10	10	8	8	10	1	2	10
N. brasiliensis																
Sl 54	−	6,000[c]	10	0	1	1	1	0	3	5	0	1	3	1	1	4
	+	3,000	10	0	1	2	2	0	5	7	0	2	5	2	2	6
IMRU 1188	−	6,000[c]	10	0	1	0	1	0	2	3	0	1	2	0	1	3
	+	3,000	10	0	2	2	0	0	4	5	0	2	3	2	1	5

[a] Inoculum used for each species corresponded to the LD_{50} values for the two groups.
[b] Dose of 7.5 mg/animal.
[c] Arbitrary amount because LD_{50} is not known (see text).

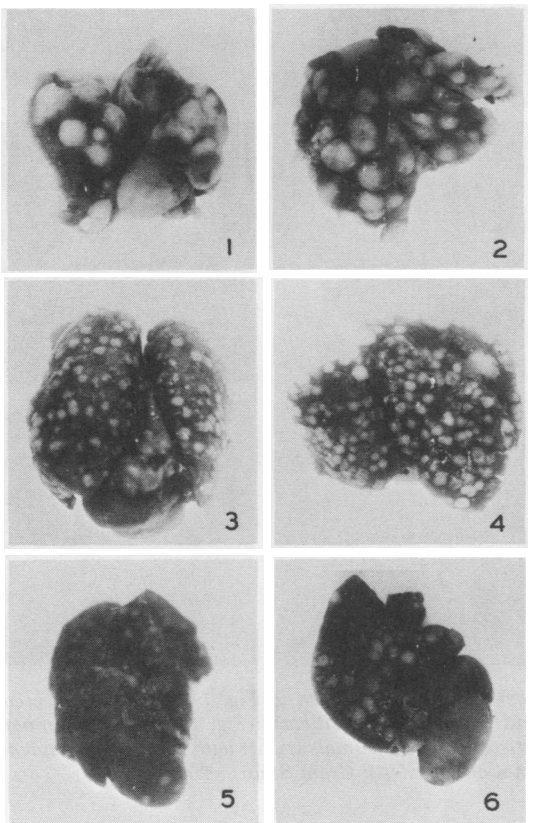

FIG. 1–6. *Gross lesions in lungs of mice dying within 2 weeks of intravenous injection with* LD_{50} *inoculum of N. asteroides Sp 27 (Fig. 1 and 2); N. caviae Sl 163 (Fig. 3 and 4); and N. brasiliensis Sl 54 (Fig. 5 and 6). Note more extensive lesions in cortisone-treated (right) than in the normal animals (left).* $\times 1.7$.

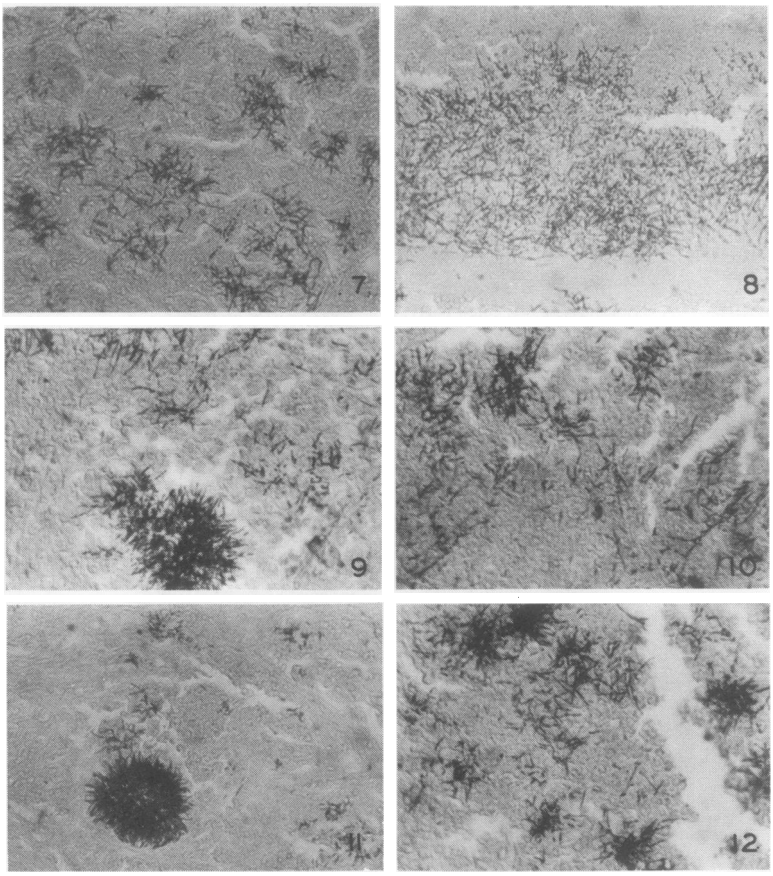

FIG. 7–12. *Sections through lung lesions (shown in Fig. 1–6) in mice infected with N. asteroides (Fig. 7 and 8), N. caviae (Fig. 9 and 10), and N. brasiliensis (Fig. 11 and 12). Compare for each species its loose and dispersed growth in cortisone-treated animals (right) with its marked tendency to produce conglomerate growth in the tissues of normal animal (left). Gram Stain. ×254.*

ance and measured 1 to 3 mm in diameter (Fig. 5 and 6). Histopathologically, they showed granules surrounded by polymorphs, lymphocytes, and monocytes. Gram-positive, thin, branching filaments of *Nocardia* were demonstrable in the granules. Some of the cortisone-treated as well as the normal mice showed active lesions when sacrificed at the end of observation period.

DISCUSSION

The present study shows that the administration of cortisone enhances the susceptibility of white mice to infection by the three species of *Nocardia* tested. This is particularly evident from the LD_{50} values of *N. asteroides* and *N. caviae* for cortisone-treated animals which were found to be one-seventh and one-eighth, respectively, of those obtained for the normal animals. The cortisone-treated mice not only suffered from much higher and more rapid mortalities but also from more extensive lesions than did the normal animals. A number of reports are available on the effect of corticosteroids on experimental infections in laboratory animals due to a variety of fungi (1, 2, 7, 8, 10, 12–14), bacteria (6, 16; see also 4), and viruses (4, 16). It appears that the foregoing data on LD_{50} values will serve as a useful aid in verification of pathogenicity of *Nocardia* species for laboratory animals.

As observed in the present investigation, cortisone administration also seems to influence the nature of microbial growth in the lesions, possibly through the suppression or altered immune response of the treated host. The degree of inflammatory response was uniformly low in the treated animals, and nocardial

growth in the lesions, particularly of *N. asteroides* and *N. caviae*, was somewhat freely dispersed, in contrast to their tendency to form conglomerate growth or even granules in the tissues of untreated animals. The low virulence of *N. brasiliensis* for laboratory animals observed here and also reported by earlier investigators (3, 5, 9) conforms to its known pattern of behavior as a pathogen of man, that is, the infections caused by this species are generally localized in the form of mycetoma and do not assume the systemic character.

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