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NOTES

Cerebral Mucormycosis in Diabetic Mice After Intrasinus Challenge

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Rhinocerebral involvement is the most common form of mucormycosis in uncontrolled diabetic patients. Streptozotocin-induced diabetic and normal mice were challenged by intrasinus inoculation of *Rhizopus oryzae* to determine their susceptibility to infection by this route. Ninety percent of diabetic mice and no normal mice died after inoculation with 10^6 spores. Intrasinus inoculations caused minimal direct trauma and low levels of brain inocula, but progressive cerebral infections were induced in diabetic mice.

Diabetes mellitus, especially with ketoacidosis, has been the most commonly recognized underlying disease associated with rhinocerebral mucormycosis (5). We have recently developed a pulmonary mucormycosis model in streptozotocin-induced diabetic mice after intranasal instillation of *Rhizopus oryzae* (A. R. Waldorf, N. Ruderman, and R. D. Diamond, submitted for publication). Unlike other models for mucormycosis (2, 6–9), there was a specific enhanced susceptibility to infection by *R. oryzae*, resembling the predisposition to mucormycosis which occurs in uncontrolled human diabetic patients. Rhinocerebral involvement is the most common form of mucormycosis in humans with diabetes; however, no experimental murine model of this specific form of the infection has been reported. Therefore, we challenged diabetic and normal mice with intrasinus inoculations of *R. oryzae* to determine their susceptibility to infection by this route.

Diabetes was induced by streptozotocin (250 mg/kg; U-9889, lot 1180K; Upjohn Co., Kalamazoo, Mich.) in 4- to 6-week-old, pathogen-free CD-1 female mice (Charles River Breeding Laboratories, Kingston, N.Y.) (1). Streptozotocin was dissolved in citrate buffer (pH 4.2). For each experiment, mice were used 7 to 14 days after streptozotocin injection. A mild ketoacidotic diabetes developed in streptozotocin-treated mice within 7 days (Waldorf et al., submitted). Control mice received 0.2 ml of buffer.

A clinical isolate of *R. oryzae* was maintained and spores were harvested as described in our previous reports (3, 9). After removal of hyphal fragments by filtration through cheesecloth, spores were washed and CFU were determined (9).

R. oryzae spores (10^6 in 0.05 ml of phosphate-buffered saline) were inoculated directly into the ethmoid sinus via the anterior nasal cavity of diabetic and normal mice using 25-gauge needles. Control mice were given sterile phosphate-buffered saline. Ninety percent (18 of 20) of diabetic mice died after intrasinus inoculation with 10^6 spores (Fig. 1). A total of 20 of 26 diabetic mice died within 4 days after challenge. In contrast, there were no deaths in normal control mice after challenge with 10^6 *R. oryzae* spores. Of 20 diabetic mice, 1 died after receiving a sterile intrasinus challenge, presumably from trauma since no bacteria were cultured.

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After mice died or were sacrificed, the organ distribution of viable fungi was determined (9). Brain and lung tissue from all diabetic mice with fatal infections invariably contained viable fungi (Table 1). Recovery of fungi was reduced in tissues of normal mice after intrasinus challenge. Moreover, in normal mice, all brain cultures were sterile.

Brain tissue from inoculated diabetic mice was fixed, sectioned, and stained by Grocott methenamine silver and hematoxylin and eosin methods. Brain lesions consisted of small unencapsulated abscesses containing hyphal elements and necrotic tissue.

Numbers of viable ungerminated *R. oryzae* spores were quantitated from groups of 10 mice. After inoculation, brains and lungs of killed animals were aseptically removed, homogenized, diluted, and plated on Sabouraud agar (A. R. Waldorf, L. Peter, and A. Polak, Sabouraudia, in press). The mean number of CFU recovered and standard errors of the natural log were calculated (4). After intrasinus challenge, only 0.004% of the inocula were recovered from brain tissue, whereas 5.6% of inocula were recovered from the lungs (Table 2). Thus, the number of *R. oryzae* CFU recovered from the brain immediately after intrasinus challenge was lower than when equal numbers of *R. oryzae* were instilled intranasally ($P \leq 0.001$; Student's t test). Comparable numbers of viable spores were recovered from lungs regardless of inoculation route. Bacterial cultures of brain and lung tissues were negative. Cardiac blood samples taken 15, 30, 60, or 120 min after intrasinus or intranasal challenge (from five mice at each time and each route) were sterile. Therefore, the larger quantity of *R. oryzae* spores recovered from brain tissue after intranasal instillations did not result from secondary inoculation caused by fungemia. Thus, it appears that intrasinus inoculations were associated with minimal local trauma causing direct intracranial spread of organisms.

TABLE 1. Spore localization in diabetic and normal mice after intrasinus inoculation[a]

Mice	No. of mice/total no.[b]					
	Deaths	Brain	Lung	Spleen	Kidney	Liver
Diabetic	20/26	10/13	12/13	5/13	3/13	2/13
Normal	1/20	0/10	4/10	3/10	1/10	2/10

[a] Intrasinus inoculation with 10^6 CFU of *R. oryzae* spores.
[b] Spore localization at the time of death or 10 days after inoculation.

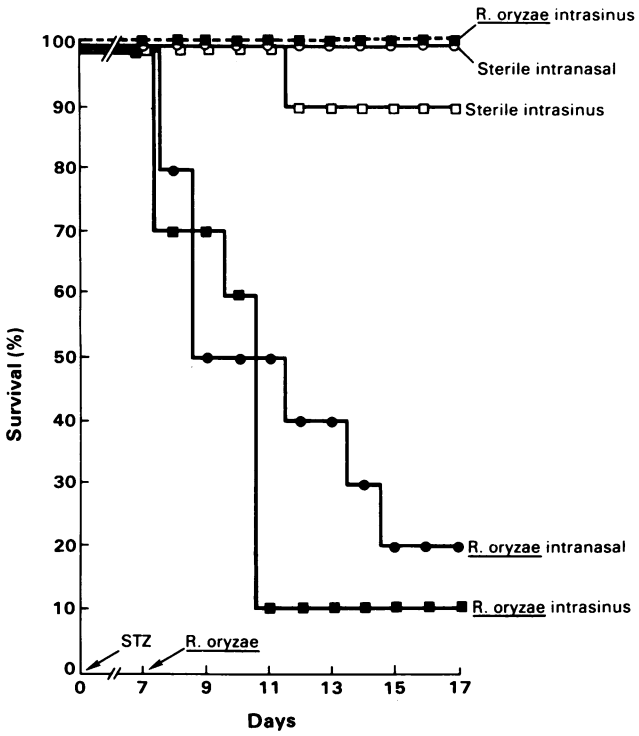

FIG. 1. Survival of normal (– – –) and diabetic (——) mice after intrasinus (■) or intranasal (●) challenge with 10^6 CFU of *R. oryzae* or sterile intrasinus (□) or intranasal inoculations (○). Streptozotocin (STZ; 250 mg/kg) was administered on day 1; *R. oryzae* or buffer was administered on day 7. $n = 20$ for each group.

In these studies, streptozotocin-induced diabetes predisposed mice to cerebral mucormycosis caused by *R. oryzae*. Intrasinus inoculation of *R. oryzae* resulted in high mortality of diabetic mice, although low numbers of organisms were recovered from brains. Although intrasinus inoculations caused minimal direct trauma and lower levels of brain inocula than the intranasal route immediately after challenge, our results suggest that progressive cerebral infections were induced in diabetic mice.

The cerebral and pulmonary model of mucormycosis in streptozotocin-induced diabetes described here and in previous reports (Waldorf et al., submitted) should prove useful in studies of the pathogenesis of mucormycosis. The route of inoculation, the specific enhanced susceptibility, and the form of mucormycosis induced should make the intrasinus infection model particularly valuable in view of its similar features to rhinocerebral mucormycosis seen in humans with diabetic ketoacidosis.

TABLE 2. Lung and brain recoveries of *R. oryzae* after intrasinus or intranasal inoculation[a]

Route	*R. oryzae* recovered (CFU)	
	Brain	Lung
Intrasinus[b]	44.2 ± 1.61[c]	$5.9 \times 10^4 \pm 1.5$
Intranasal[d]	$1.4 \times 10^3 \pm 2.4$	$1.7 \times 10^4 \pm 1.9$

[a] Inoculation with 5×10^6 CFU of *R. oryzae*.
[b] Animals were sacrificed within 5 min of challenge.
[c] $P \leq 0.001$.
[d] Animals were sacrificed within 1 to 2 h of challenge.

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