

Invasive aspergillosis - a rabbit model

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Abstract

An invasive aspergillosis model in rabbits was attempted using 3 concentrations of *A. fumigatus* conidia. Conidia concentrations of 1×10^6 , 1×10^7 and 1×10^8 were inoculated intravenously into rabbits. The severity of infection was directly proportional to the inoculum size of the conidia. *Aspergillus fumigatus* was isolated from livers, kidneys, spleens, hearts and lungs of infected rabbits at a rate of 82%, 75%, 57%, 54% and 32% respectively. Cultures of urine specimens taken by bladder cap were positive for *A. fumigatus* in 30% of the rabbits tested. Blood cultures using the Bactec Fungal System (Becton Dickinson Corp., USA) failed to isolate *A. fumigatus* in 20 rabbits with biopsy-proven invasive aspergillosis. Active infection with high fungal tissue burden occurred between 2-4 days after infection in rabbits inoculated with 1×10^7 conidia.

Key words: Aspergillosis, rabbit

INTRODUCTION

Invasive aspergillosis is a life threatening opportunistic infection in the immunocompromised host. Ante-mortem diagnosis of invasive aspergillosis by conventional methods is not helpful: cultures of blood and urine are mostly negative^{1,2} and sputum is positive for *Aspergillus* species in only 8-34% of cases.^{1,3} Early diagnosis of invasive aspergillosis is dependent on the detection of infection specific antigens in the circulation.⁴ However, most serological tests developed for antigen detection are not available commercially. A latex agglutination kit, Pastorex Aspergillus, (Institute Pasteur, France) which detects the galactomannan antigen of the *Aspergillus* has been marketed. This kit is reported⁵ to detect the galactomannan antigen at a minimum level of 15ng/ml and to have high sensitivity and specificity. However, from our experience, this kit demonstrates false positivity if serum specimens contain trace amounts of red blood cell lysis or if the sera is conserved in the blood clot prior to its separation. Additionally, this kit is very expensive. These shortcomings limit the value of the latex agglutination kit. There is a need to develop a more reliable and cheaper serological technique for aspergilli antigen detection. However, immunodiagnostic studies are dependent on the availability of specimens from proven clinical cases of invasive

aspergillosis or specimens from animal models. Since the former is difficult to obtain, animal models of the infection have to be established. The aim of this study is to establish an invasive aspergillosis model using rabbits which can be used for immunodiagnostic studies in the future. In this study, 3 concentrations of *Aspergillus fumigatus* conidia were used to infect rabbits. The relationship of the 3 doses of *A. fumigatus* conidia to the severity of infection in these rabbits was also investigated.

MATERIALS AND METHODS

Microorganism

Aspergillus fumigatus M175/85 used in this study was isolated from a tracheal aspirate of a diabetic patient with pulmonary abscesses and who was not responding to antibiotics.

Inoculum preparation

Conidia of *A. fumigatus* M175/85 were harvested by washing 6 day old Malt Agar cultures of *A. fumigatus* with 0.1% Tween 80 made up in 0.8% NaCl. The fungal suspension was then filtered aseptically through 4 layers of gauze and vortexed vigorously with glass-heads in order to obtain a suspension of singly dispersed conidia of *A. fumigatus*. The concentration of viable conidia was determined by culture. The

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viability of the conidial suspension was not affected when kept at 4°C for 1 week.

Rabbit model

Thirty local female rabbits weighing 1.8- 2.8kg were used. Rabbits were divided into 4 groups, A, B, C and D. Rabbits in groups A, B and C were injected through the lateral ear vein with viable conidia of *A. fumigatus*. Twelve rabbits (No.1 to 12) in group A received 1×10^6 viable conidia in 0.5ml of NaCL-Tween 80 solution. Another 12 rabbits (No.13 to 24) in group B received 1×10^7 viable conidia and 4 rabbits (No.25 to 28) in group C received 1×10^8 viable conidia. Two rabbits (No.29 to 30) in group D, which constituted the control group, were injected intravenously with 0.5ml of NaCL-Tween 80 solution. The rabbits were observed for a period of 28 days. In group A, 2 rabbits at a time were sacrificed by cutting the bilateral jugular vein at 2, 4, 7, 14, 21 and 28 days post-infection. All rabbits in this group had remained healthy by gross appearance at the times of sacrifice. Rabbits in group B and C were sacrificed when they appeared moribund. Moribund rabbits were weak and non-agile. Some rabbits in group B and C also died as a result of the infection. Necropsies were conducted on all rabbits that were sacrificed or that died. Representative portions of the liver, kidney, spleen, heart and lungs that appeared infected were selected, weighed and finely ground with a mortar and pestle. Dilutions of the tissue suspension were plated out in duplicates onto Saubouraud Dextrose Agar containing antibiotics (Oxoid) and cultured at 37°C for 1 week. During animal experimentation, the institution's guidelines for laboratory animal care was adhered to.

Blood culture

Blood from 22 rabbits (2 controls and 20 infected rabbits) were collected by intracardiac puncture just before sacrifice. Out of the 20 infected rabbits from which blood was collected, 12 were from group A (1×10^6 conidia), 7 from group B (1×10^7 conidia) and 1 from group C (1×10^8 conidia). Immediately after collection, 10 ml of blood was inoculated into Bactec Fungal system (Becton Dickinson, Corp. The bottles were incubated with aeration at 37°C for 1 week. Two readings a day for 1 week were taken using the NR 730 system (Becton Dickinson, Corp., USA). Readings of 30 and above were considered positive for aspergilli growth. A week after culture, Bactec Fungal

systems were opened aseptically and subcultured onto Saubouraud Dextrose Agar at 30°C for another week.

Bladder tap culture

Bladder tap procedures were conducted on 10 rabbits: 1 rabbit from group A, 6 from group B and 3 from group C. The urine samples obtained were centrifuged at 4,000g for 10 min and the sediments cultured onto Saubouraud Dextrose Agar for 1 week at 37°C.

RESULTS

Progression of infection

The progression of infection in the rabbits was assessed by the rate at which the rabbits were moribund or died throughout the course of infection (Fig. 1). Of rabbits inoculated with 1×10^8 conidia, 50% and 100% were moribund or dead by days 2 and 4 post-infection respectively. In the group that received 1×10^7 conidia, 50% and 100% of the rabbits were moribund or dead by days 3 and 11 post-infection respectively. All rabbits which were given 1×10^6 conidia remained healthy for the whole duration in which the animals were maintained.

Isolation of A. fumigatus

Table 1 demonstrates the frequency in which *A. fumigatus* were cultured from organs, urine and blood of rabbits inoculated with *A. fumigatus* conidia. Rabbits injected with 1×10^8 conidia demonstrated 100% recovery of *A. fumigatus* from liver, kidney, spleen and heart. In rabbits that received 1×10^7 conidia, 100% isolation of *A. fumigatus* was obtained in the livers and kidneys only. *Aspergillus fumigatus* was most frequently isolated from the liver, followed by kidney, spleen, heart and lung of all infected rabbits. The overall rates of isolation of *A. fumigatus* from liver, kidney, spleen, heart and lung of all inoculated rabbits were 82%, 75%, 57%, 54% and 32% respectively. All blood cultures were negative for *A. fumigatus*. Culture of urine taken by bladder tap procedure were positive for *A. fumigatus* in 3 out of 10 rabbits: 1 that was inoculated with 1×10^7 conidia and 2 inoculated with 1×10^8 conidia. Tissues and blood from control rabbits which were uninoculated were negative for *A. fumigatus*.

Fungal tissue burden

Aspergilli load in tissues was greatest in rabbits

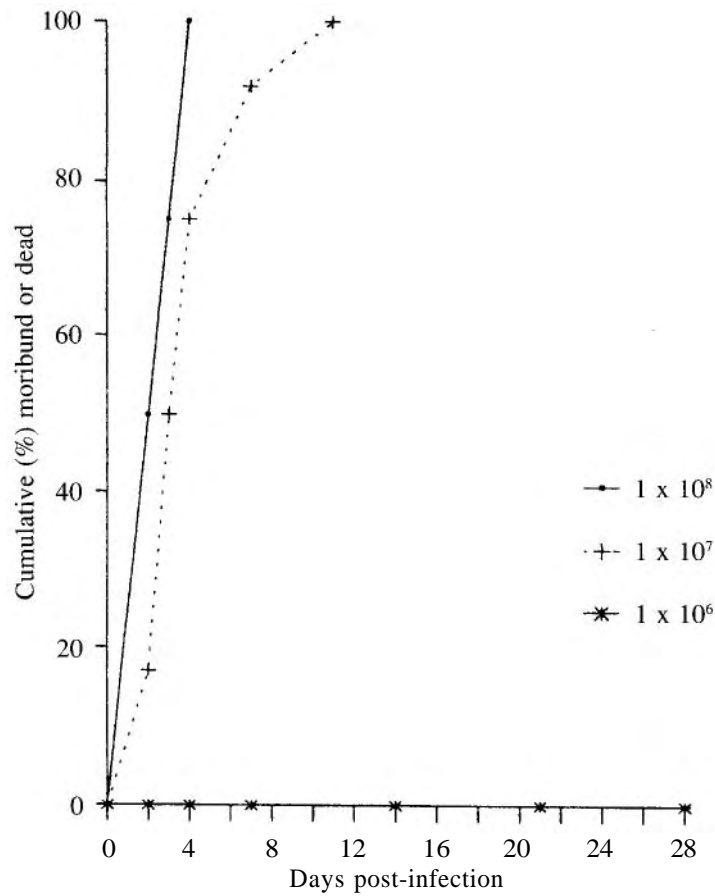


FIG. 1: Progression of infection in rabbits inoculated intravenously with 3 concentrations of viable *Aspergillus fumigatus* conidia.

TABLE 1: Isolation of *Aspergillus fumigatus* from rabbits infected intravenously with viable conidia

Organs/fluids	Rabbit group			
	No. organs positive/ Total no. cultured (%)			
	All rabbits	A	B	C
Liver	23/28 (82)	7/12 (58)	12/12 (100)	4/4 (100)
Kidney	21/28 (75)	5/12 (42)	12/12 (100)	4/4 (100)
Spleen	16/28 (57)	3/12 (25)	9/12 (75)	4/4 (100)
Heart	15/28 (54)	3/12 (25)	8/12 (67)	4/4 (100)
Lung	9/28 (32)	2/12 (17)	4/12 (33)	3/4 (75)
Urine	3/10 (30)	0/1 (0)	1/6 (17)	2/3 (67)
Blood	0/20 (0)	0/12 (0)	0/7 (0)	0/1 (0)
Average % positive		33.3	75	95

Group A: Rabbits inoculated with 1 x 10⁶ conidia
 Group B: Rabbits inoculated with 1 x 10⁷ conidia
 Group C: Rabbits inoculated with 1 x 10⁸ conidia

TABLE 2 : *Aspergillus fumigatus* load in tissues after 1×10^6 , 1×10^7 and 1×10^8 viable conidia inoculation into rabbits.

Days post-infection/Concentration of viable conidia inoculated/CFU (log ₁₀) per gram of tissue.													
Organs	2			4			7		11		14	21	28
	1×10^6	1×10^{7a}	1×10^{8b}	1×10^6	1×10^7	1×10^8	1×10^6	1×10^7	1×10^6	1×10^7	1×10^6	1×10^6	1×10^6
Liver	1.93	3.45	6.30	2.41	3.59	6.54	2.16	3.29	ND ^c	2.42	1.41	0	0
Kidney	1.32	2.19	5.38	1.65	3.08	5.62	0	2.11	ND	2.34	1.66	0	0
Spleen	1.30	1.70	4.79	0.48	3.13	5.92	0	0.48	ND	2.45	0	0.6	0
Heart	0.70	2.05	5.92	0.48	0.78	1.04	1.56	0.60	ND	0.30	0	0	0.6
Lung	1.84	0.70	7.60	0	0.48	4.52	0	0	ND	0	0	0	0
Rabbit	1,7	13,14	25,26	3,4	21,22,23	28	5,6	15,17	20	7,8	9,11	10,12	

a All rabbits inoculated with 1×10^7 viable conidia were sacrificed or died by day 11 post-infection.

b All rabbits inoculated with 1×10^8 viable conidia were sacrificed or died by day 4 post-infection.

c ND = not done.

infected with 1×10^8 conidia, followed by rabbits infected with 1×10^7 and 1×10^6 conidia (Table 2). In rabbits infected with 1×10^7 conidia, the highest fungal load in liver, kidney and spleen were detected in rabbits sacrificed at the 4th post-infection day. A rabbit that received 1×10^8 conidia and which died at day 4 post-infection demonstrated at least 10^4 fold less aspergilli load in the heart than other rabbits given the same dose that died earlier.

DISCUSSION

The severity of *A. fumigatus* infection in rabbits was found to be directly proportional to the inoculum dose of viable conidia. When the inoculum dose was increased 10 fold serially from 10^6 to 10^8 the rate at which rabbits were moribund or dead and the frequency of fungal isolation in tissues also rose. The fungal load was also greater in rabbits injected with higher concentrations of conidia than lower.

Amongst the tissues examined for infection, the liver and kidney of the rabbits were found to be the main target organs of *A. fumigatus*. In contrast, the lung was the organ least infected; it had an overall isolation rate of 32% compared to 82% for liver and 75% for kidney. The low isolation rate from the lungs could be due to the efficient ingestion and destruction of the inoculated conidia by alveolar macrophages as it has been reported that alveolar macrophages can destroy conidia without any need for oxidative

burst of T cell modulation.⁶ Although *A. fumigatus* was readily isolated from the various types of tissues examined, none was isolated from blood samples. This could be due to their absence, or the isolation technique used was not suitable for the recovery of *A. fumigatus*. It has been reported that the isolation of this fungus from blood is extremely difficult.^{1,2,3}

Although gross pathological changes in infected animals were widespread and clearly seen as microabscesses in the liver; kidney, spleen and lung, they were less noticeable in the heart. Therefore, samples of heart tissue with different levels of infection might have accounted for the observation of a vast difference in fungal load in the hearts of 2 rabbits from the same inoculum group.

When the selection of one of the 3 conidia inoculum doses used in this study were considered for establishing an invasive aspergillosis model which could be used in immunodiagnostic studies, the dose of 10^8 viable conidia per rabbit was considered not suitable as the progression of infection leading to death was too rapid. It was felt that a dose of 10^7 was more suitable as at this dose the rabbits were highly infected but that the progression of fatal infection was slower than rabbits inoculated with 10^8 conidia. Therefore, the use of this dose would create a wider period of time in which infection with high fungal tissue load can be examined before the rabbits died. In rabbits inoculated with 1×10^7 conidia, the concentration of aspergilli in

tissues was high between 2 - 4 days post-infection. It was also during this interval of time that 75% of rabbits were moribund or dead. Weiner and Coats-Stephen⁷ demonstrated the highest tissue load of *Aspergillus* and greatest death rate between 2-5 days of infection. Antigenaemia in invasive aspergillosis which correlates with disease activity is also most likely to be detected at 2-6 days post-infection."''''''

Rabbits which were infected with 1×10^7 conidia seemed to demonstrate the progression of infection which is most suitable for immunodiagnostic studies. Future work will concentrate on detection of antigens in sera of rabbits which are similarly infected.

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