Initial organ localisation of blood-borne *Candida albicans* in a rat model of disseminated candidosis

S. KATZ,* J. L. GROSFELD, W. J. FOLKENING,† R. S. ROSENTHAL† and G. J. MERKEL‡

Department of Surgery, Section of Pediatric Surgery, James Whitcomb Riley Hospital for Children, Indianapolis.

† Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, and

‡ Department of Microbiology and Immunology, Indiana University School of Medicine, Fort Wayne, IN 46805, USA

Summary. The rat was evaluated as an experimental model for disseminated candidosis by quantitating blood clearance and initial organ localisation of ³H-leucine-labelled *Candida albicans* after intravenous injection into the tail or portal vein. Viable or formalin-killed blastoconidia or viable blastoconidia with germ tubes were injected into experimental animals. Blood and tissue samples were obtained up to 24 h after injection and processed for liquid scintillation counting (to determine the distribution of labelled yeasts) and quantitation of viable organisms. Yeasts were cleared rapidly after intravenous (i.v.) injection by either route, i.e., < 5% of the radioactivity was detected in the blood after 5 min. The liver and lung were the major organs that sequestered blood-borne yeasts 1 h after tail vein injection (42.5 SD 15% and 41.4 SD 6.4% of labelled yeasts injected, respectively). However, injections *via* the portal vein resulted in trapping of the yeasts predominantly by the liver. Recovery of radioactivity and viable yeasts from all organs except the kidneys decreased with time. Overall, the results indicated that the rat might serve as a reliable model for short-term studies on organ distribution and thus contribute to our understanding of tissue tropism in candidosis.

Introduction

Normally a harmless commensal of the gastrointestinal tract, oropharynx, skin and female genital tract, *Candida albicans* may convert to a life-threatening pathogen in predisposed patients. These include neonates, immunosuppressed and post-operative patients and those undergoing prolonged intravenous catheterisation, hyperalimentation and broad-spectrum antimicrobial chemotherapy.^{1–3}

Potential virulence factors of *C. albicans*, such as morphogenesis and production of extracellular hydrolytic enzymes, continue to be investigated, but there is limited knowledge of the initial fate of blood-borne yeasts, as would be encountered during fungaemia.^{4–6} Blood clearance and organ localisation have been studied in mice,^{7–9} rabbits,¹⁰ dogs^{4,5} and guinea-pigs,¹¹ but the studies were either conducted over an extended period or involved quantitating yeasts in various tissues by radioactive measurements or viable cell counts, but not both methods together. Utilising both would appear beneficial since the

radioactivity values indicate the total amount of organ trapping whereas the viable cell counts indicate additionally the extent of yeast survival and growth in a particular tissue.

Rats are useful models for studies on mucocutaneous,^{12–15} urinary tract,^{16,17} and gastrointestinal¹⁸ candidosis, but have seldom been used to investigate blood clearance and organ localisation during fungaemia. Because it would be beneficial to establish one animal model for studies on all phases of candidosis, we evaluated the rat as a model for blood clearance and initial organ localisation of radiolabelled *C. albicans* clinical isolates. We have previously studied the organ localisation of *C. albicans* in experimentally-induced obstructive jaundice in rats¹⁹ and also the in-vitro interaction of *C. albicans* with rat cells in monolayer cultures.^{20,21} This report continues our investigations *in vivo* and compares the distribution of radiolabelled yeasts to the recovery of viable yeasts after intravenous (i.v.) injection into the tail and portal veins of rats.

Materials and methods

Experimental animals

Male Sprague-Dawley rats (Harlan SD Inc., Indianapolis, IN, USA) weighing 175–180 g were housed four/cage and fed standard rodent laboratory chow

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* Present address: Department of Pediatric Surgery, Hadassah University Hospital, PO Box 12000, Jerusalem, Israel 91120.

‡ Correspondence should be sent to Dr G. J. Merkel, Department of Microbiology and Immunology, School of Medicine, Indiana University School of Medicine, Fort Wayne Center for Medical Education, 2101 Coliseum Blvd East, Fort Wayne, IN 46805, USA.

(Purina Mills Inc., St Louis, MO, USA) and tap water *ad libitum*. Rats were placed in experimental groups according to the route of injection (tail *versus* portal vein), the clinical isolate of *C. albicans* employed, and whether the yeasts were viable or formalin-killed blastoconidia, or blastoconidia with germ tubes.

Yeast isolates and culture conditions

Strains of *C. albicans* were isolated from clinical specimens derived from different patients and identified by standard techniques.²⁰ The strain designations and their origins were as follows: PV-2, tracheal aspirate (bronchopulmonary candidosis); PV-8, suture (wound infection); and PV-25, tongue (oral thrush). Yeast stock cultures were maintained as described previously.^{20,21} Radiolabelled yeasts used in the experiments outlined below were prepared by growing the yeasts for 10 h (early stationary phase) at 37°C on a rotary shaker in Yeast Nitrogen Base (Difco) containing glucose 0.25%, (NH₄)₂SO₄ 0.5% and L-(4,5-³H) leucine (Amersham, Arlington Heights, IL, USA) 2 µCi/ml. The radiolabelled yeasts were washed twice with NaCl 0.9%, cell numbers were determined microscopically with a haemocytometer, and the suspension was adjusted to 1 × 10⁷ blastoconidia/ml. The final suspensions typically had a specific activity of 1 ³H dpm/100–1000 blastoconidia.

Formalin-killed yeasts were prepared by treating radiolabelled blastoconidia, grown as described above, with formalin 10% in phosphate buffered saline (PBS, pH 7.2) for 15 min at 5°C. These yeasts were harvested by centrifugation and washed twice with NaCl 0.9% before injection. Complete killing of the yeasts by this technique was confirmed by inoculating Sabouraud Dextrose Agar (SDA, Difco) with 1 ml of the resulting yeast suspension.

Blastoconidia with germ tubes were prepared after growing the yeasts as described above, washing once with NaCl 0.9%, and incubating the yeasts (1 × 10⁷/ml) at 37°C for 1 h in Dulbecco's modified Eagle medium containing fetal bovine serum 5% as described previously.²¹ These yeasts were harvested by centrifugation, washed twice with NaCl 0.9% and passed through a 26-gauge needle before injection. These procedures yielded suspensions of individual blastoconidia of which > 90% had germ tubes 1–2 times the diameter of the parent cell in length. Aggregates were not observed microscopically.

Determination of viable yeasts in organ samples

Tissue samples (c. 0.1 g each) were weighed and suspended in PBS. After tissue sections had been taken for viability and radioactivity determinations, the entire organs were weighed. The tissue samples were then homogenised in glass dounce homogenisers (Fisher Scientific, Itasca, IL, USA), the resulting tissue homogenates were diluted appropriately with PBS (1

in 10 and 1 in 100) and samples (0.1 ml) were plated on to SDA. The inoculated plates were incubated at 37°C for 48 h before colonies were counted. The results were expressed as the mean colony forming units (cfu)/intact organ relative to the total number of cfu injected. A specific problem associated with determining the number of viable yeasts by the plate count method is the tendency of *C. albicans* to aggregate. The lack of aggregation in the initial yeast suspensions was confirmed microscopically. However, it was not possible to monitor aggregation which occurred *in vivo*.

Blood clearance and organ localisation of radiolabelled yeasts

Experimental animals were sedated with intramuscular injections of ketamine cocktail (Aveco, Ft Dodge, IO, USA), composed of (/ml) ketamine 100 mg, promazine 2.2 mg and atropine 0.4 mg; then 1 ml of radiolabelled yeasts (1 × 10⁷ blastoconidia) was injected *via* a lateral tail vein with a 19-gauge Butterfly® infusion set (Abbott Hospitals Inc., North Chicago, IL, USA) or *via* the portal vein by mid-laparotomy. Haemostasis was achieved by direct pressure for 3 min.

To evaluate blood clearance of the radiolabelled yeasts, rats were sedated, the tail was transected, and 50 µl of blood was obtained from actively bleeding proximal tail vessels with heparinised micropipettes. These blood samples were processed for scintillation counting as described below.

To evaluate organ localisation of injected radiolabelled yeasts, rats were sedated as before, tissue samples (0.05–0.10 g each) were collected at various times up to 24 h after injection, weighed and placed into scintillation vials. Whole organs were weighed after all samples were collected from a particular animal. Tissue samples for radioactivity measurements were dissolved in 1 ml of Soluene 350 (Packard Camberra Co., Downers Grove, IL, USA) overnight at 50°C. Isopropanol and H₂O₂ 30% were then added until the digests clarified. After incubating the samples for 30 min, 1 ml of 5 N HCl followed by 15 ml of Insta-Gel X-F scintillation cocktail (Packard) were added. Radioactivity was determined with a 2200 CA Tricarb liquid scintillation analyser (Packard) interfaced with an IBM microcomputer. Data were corrected for quench by use of a calibration curve established by external standardisation for ³H. The number of ³H dpm in individual samples was converted to dpm/intact organ and the mean dpm/organ was expressed as the percentage of the total dpm injected. The statistical significance between some of the results was evaluated by one-way analysis of variance (ANOVA).

Results

Blood clearance

Radiolabelled yeasts were cleared rapidly from the

blood and within 5 min after injection *c.* 4% of the original inocula (dpm) were recovered from blood samples (calculation based on blood volume being *c.* 15% of total body weight). After incubation for 1 and 2 h, *c.* 1.4% and 1.7% of the inocula were recovered, respectively. Blood clearance rates were independent of the yeast isolate used, of culture viability (*i.e.*, untreated or formalin-killed blastoconidia) and of the presence or absence of germ tubes.

Organ localisation of radiolabelled yeasts

Fig. 1 shows the organ localisation of viable radiolabelled *C. albicans* (PV-8) blastoconidia after tail vein injection. After 1 h, most of the radioactivity was localised in the liver and lungs (42.5 SD 15% and 41.4 SD 6.1% of the amount injected, respectively). After 4 h there was a decrease in the radioactivity recovered from lung tissue but not from liver. A decrease in radioactivity was apparent in both organs after 24 h. The organ localisation and recovery of radioactivity from rats given the PV-2 or PV-25

Fig. 1. Organ localisation and recovery of radioactivity after injection of *C. albicans* PV-8 via the tail vein; values are based on the dpm/organ recovered from the liver (○—○), lung (●—●), spleen (△—△) and kidney (▲—▲).

Table. Organ localisation after injection of viable and formalin-killed blastoconidia and blastoconidia with germ tubes

Time (h)	Yeast injected	Mean (SD) percentage dpm injected/intact organ†			
		Kidney	Spleen	Liver	Lung
1	Viable	1.6 (0.4)	3.8 (1.0)	42.5 (15.0)	41.4 (6.4)
	Killed	1.8 (0.3)	4.7 (0.2)	51.6 (12.0)	41.2 (11.1)
	Germinated	2.5 (0.3)	3.0 (0.6)	36.3 (7.9)	53.7 (7.1)*
4	Viable	1.5 (0.3)	4.3 (0.6)	44.0 (8.0)	23.9 (5.5)
	Killed	1.2 (0.3)	3.3 (1.0)	37.8 (8.0)	14.3 (2.1)*
	Germinated	2.3 (0.3)	4.2 (1.0)	43.4 (5.4)	26.9 (5.4)
24	Viable	1.3 (0.2)	2.0 (0.6)	20.4 (4.2)	3.7 (1.3)
	Killed	0.8 (0.5)	2.1 (0.4)	18.9 (4.8)	1.9 (0.7)
	Germinated	1.2 (0.2)	1.8 (0.4)	17.9 (6.7)	2.3 (0.6)

* ANOVA, $p < 0.005$ compared to values obtained with viable blastoconidia.

† 20 rats in each test group.

Fig. 2. Organ localisation and recovery of viable *C. albicans* PV-8 after tail vein injection; values are based on the cfu/organ recovered from the liver (○—○), lung (●—●), spleen (△—△) and kidney (▲—▲).

isolates (data not shown) were not significantly different from those reported for the PV-8 isolate.

The organ distributions of radioactivity from rats given PV-8 blastoconidia, formalin-killed blastoconidia and blastoconidia with germ tubes are summarised in the table. Generally, the condition of the injected yeasts had little effect on the recovery and distribution of radioactivity. There was no consistent pattern to the few statistically significant differences evident.

Organ localisation based on viability results

The distribution and recovery of viable blastoconidia after tail vein injection are shown in fig. 2. In contrast to the recovery of radioactivity, more than twice the number of viable yeasts were recovered from liver samples than from the lungs 1 h after injection. The recovery of viable yeasts from all organs except the kidneys decreased with time and after 24 h the concentration of viable yeasts in the kidneys increased to levels similar to those recovered from the liver. The recovery and distribution of viable yeasts were unaffected by the isolate of *C. albicans* injected or whether they were blastoconidia or blastoconidia with germ tubes (data not shown).

Organ localisation after portal vein injection

The recovery of radioactivity from the lungs and liver after injection of viable blastoconidia (PV-8) via the portal and tail veins are compared in fig. 3. Portal vein injection resulted in the majority of the radioactivity being trapped in the liver. However, after 24 h similar recovery and organ distribution were observed regardless of the route of injection.

Discussion

This study, unlike those conducted with other

Fig. 3. Comparative recovery of radioactivity from the lungs and livers after injection of *C. albicans* PV-8 via the tail and portal veins; values are based on the dpm/organ recovered from the liver after injection via the portal (○—○) or tail (●—●) veins, and from the lung after injection via the portal (▲—▲) or tail (△—△) veins.

animal models,^{4-11,22} describes blood clearance and initial organ localisation of *C. albicans* by measurements of both radioactivity and viable yeasts recovered from organ samples up to 24 h after i.v. injection. The work of Stone *et al.*,^{4,5} for example, showed in a qualitative manner the capacity of various tissues to trap circulating *C. albicans*. However, their results demonstrated trapping by the lung only at very high yeast concentrations. Our data, on the other hand, suggest that, in the rat, both lung and liver tissue are quite efficient at sequestering blood-borne yeasts. Although similar amounts of radioactivity were recovered from these organs, the number of viable yeasts in lung tissue was consistently two-fold lower than the number recovered from the liver. The fact that lower amounts of viable yeasts were recovered than predicted from the recovery of radioactivity may have been related to tissue-associated yeast killing or the tendency of *C. albicans* to aggregate, or both.⁴ Although aggregation of the yeasts would cause lower than expected numbers of cfu with the plate count technique, we expect that the extent of aggregation would not be greater in lung tissue. Thus, the lung may be more efficient at killing yeasts than the liver, and the presence of live or dead *C. albicans* in this tissue might stimulate the infiltration of phagocytic cells, ultimately resulting in the formation of micro-abscesses or focal areas of necrosis.¹ The possibility of this occurring supports the use of both radioactive and viability measurements when examining organ localisation of blood-borne yeasts. Thus, the radioactive determinations estimated the extent or organ trapping of radiolabel (viable, dead, or fragments of yeasts) and the viability determinations indicated the tissue sites where yeast cells may survive or grow. This was particularly true for the kidney where it was shown that relatively small amounts of radiolabelled yeasts were sequestered, but that those trapped were apparently able to grow as shown by the increase in viable yeasts recovered after 24 h.

Evans and Mardon⁷ described organ localisation in pre-leukaemic mice with ATCC strains of *C. albicans* grown at room temperature. In their experimental system they found little difference between the amount of recovered radioactivity and the amount of viable yeasts. Furthermore, they reported that the pseudo-hyphal form was trapped more by the lung than was the yeast morphology. This is in contrast to our results which demonstrated a nearly identical pattern of fungal distribution after injection of viable blastoconidia, killed blastoconidia and blastoconidia with germ tubes. Our results implied that, in the rat model, organ localisation of blood-borne yeasts was associated with a tissue specificity that was unaffected by the condition of the yeasts. Whereas organ localisation might be related to specific receptors in the tissue for *C. albicans*, the size of the yeast cell and yeast aggregates may in part explain initial trapping of blood-borne yeasts in capillaries of the lung and liver. Additionally, the increased sequestering of *C. albicans* by the liver following portal vein injection further supports the hypothesis that yeasts entering the blood from the gastrointestinal tract may be readily trapped by the reticulo-endothelial system of the liver.^{4,6,23}

The increase in the concentration of yeasts in the kidneys after 24 h appears to have resulted from growth *in vivo*. These results are not surprising, as in longer term experimental infections the kidneys are usually the most heavily infected tissue.²⁴ Louria *et al.*⁹ were one of the first groups to conduct long term studies on organ localisation of *C. albicans* in mice and they were the first to demonstrate conclusively that the kidney was the only organ to allow growth of the yeast. Their conclusions were based on determining the concentration of viable organisms from various tissues 2-7 days after inoculation. However, short-term studies combining radioactivity and viability measurements were not conducted.

In conclusion, it appears that the rat may serve as a reliable model for tissue tropism studies with *C. albicans*. Many similarities between our studies and those of others in different species were apparent, but use of two methods for quantitating yeasts indicated that the rate of killing within different organs may be different. The trapping of circulating yeasts by certain organs might result in local or systemic inflammatory reactions or, during immunocompromising conditions, yield focal areas of infection.¹ Although *C. albicans* produces no recognised exotoxin, secreted acid proteases,^{25,26} phospholipases²⁷ or certain cell-wall components^{28,29} may contribute to the development of focal lesions in organs that sequester circulating yeasts.

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Microbial Cell Surface Analysis. Structural and Physicochemical Methods

Edited by N. MOZES, P. S. HANDLEY, H. J. BUSSCHER and P. G. ROUXHET. 1991. ISBN 3-527-27987-3. VCH Verlagsgesellschaft mbH. Pp. 368. £58.00.

This textbook reviews the methods currently available for investigating microbial cell surfaces. Some of the techniques described have been developed only recently and their inclusion reflects the comprehensive nature of the text. The principles and practical aspects, together with the application of the techniques and a useful critique are also described. These will be of particular value to workers in this field.

The first part of the book deals with the current knowledge of the cell wall structure and other related components. The significance of some of the findings is also presented. In the next section, the characterisation of the cell surface by various electronmicroscopy techniques, including freeze etching and drying, freeze substitution, and immunolabelling, is described. Negative staining and carbohydrate detection are also presented in detail. Electronmicrographs

are used to illustrate the important structural findings and the value of each of these techniques.

The third part of the book presents methods for determining atomic and molecular composition of the bacterial cell surface. There are also sections on the physical and chemical properties, such as electrical charge, hydrophobicity and surface energy. These areas are notoriously complicated, but the authors have managed to present the information in a concise but lucid fashion. The last section contains studies combining both physical, chemical and structural methods, which are used to investigate the cell surface in relation to adhesion.

The various sections in the book are obviously written by experts in cell wall surface analysis. The text is presented in a logical and well-structured manner and is illustrated with excellent figures and diagrams which augment the comprehensive nature of the text. The book was certainly interesting to read and is, without doubt, one of the best texts available on bacterial cell wall structure and it should therefore have a wide appeal.

T. S. J. ELLIOT