# *IN VITRO* AND *IN VIVO* ANTI-*CANDIDA* ACTIVITY AND TOXICOLOGY OF LY121019

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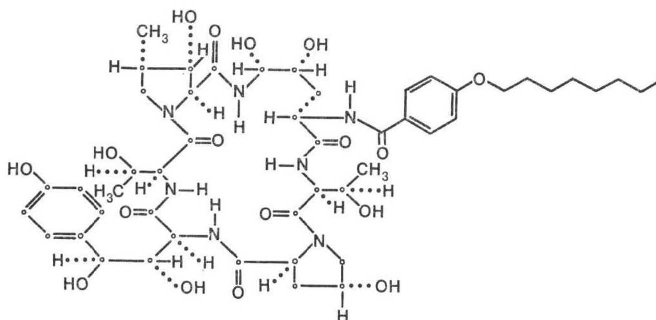
LY121019 (*N-p*-octyloxybenzoylechinocandin B nucleus) is a semisynthetic antifungal antibiotic that possesses potent anti-*Candida* activity. The $MIC_{50}$ and the $MIC_{90}$ for both LY121019 and amphotericin B were 0.625 and 1.25 $\mu$g/ml, respectively. Only an 8-fold increase in the MIC against *C. albicans* occurred during 34-day exposure to subinhibitory concentrations indicating that LY121019 has a low potential for causing resistance development. Scanning electron microscopic studies revealed that LY121019 caused severe damage to the *C. albicans* cell. The $ED_{50}$'s for LY121019 and amphotericin B administered parenterally to mice were 7.4 and 2.5 mg/kg, respectively. Parenterally administered LY121019 at doses of 6.25 mg/kg significantly reduced the recovery of *C. albicans* from infected mouse kidneys. Orally administered 50 and 100 mg/kg doses of LY121019 were effective in eliminating *C. albicans* from the gastrointestinal tract of infected mice. Topical application of 5% LY121019 was as effective as 3% nystatin in the treatment of superficial *C. albicans* infections. Local administration of LY121019, nystatin, or miconazole was effective against rat vaginal candidiasis. LY121019 was administered intravenously to dogs at doses up to 100 mg/kg/day, 5 days a week for 3 months; all dogs survived. Compound related effects included a histamine-like reaction, increased serum alkaline phosphatase and SGPT, fatty vacuolization of the liver, and some tissue damage at the injection site. The no effect dose in dog was 10 mg/kg. LY121019 had no more than 1/20 the toxicity of amphotericin B in the dog.

Echinocandin B and aculeacin are related polypeptide antifungal antibiotics with a narrow spectrum of antifungal activity although highly active against yeasts[1,2]. We are reporting on the anti-*Candida* activity and toxicology of LY121019, a novel analog of the polypeptide antifungal antibiotic echinocandin B (Fig. 1). The study involves comparison of the *in vitro* and *in vivo* anti-*Candida* activity and toxicology of LY121019 with these qualities of clinically used parenteral and topical antifungal agents.

Fig. 1. Structure of antifungal antibiotic LY121019.



## Materials and Methods

### Antifungal Agents

LY121019 was prepared in the Lilly Research Laboratories. Amphotericin B and nystatin were obtained from E. R. Squibb and Sons. Miconazole was obtained from Janssen Pharmaceuticals. Clotrimazole was obtained from the Schering Corporation. 5-Fluorocytosine was obtained from Hoffmann-La Roche.

### *In Vitro* Susceptibility Studies

*Candida albicans* isolates were grown in Sabouraud's dextrose or Yeast Nitrogen Base (YNB) medium at 30°C. The procedures used for the agar dilution and broth dilution susceptibility tests were described previously[3]. MIC determinations were made at 48 hours.

### *In Vitro* Resistance Development

Tubes containing 5 ml YNB broth and doubling concentrations of LY121019 from 0.039~80 μg/ml were prepared in triplicate. Each tube was inoculated with $1\times10^4$ log phase *C. albicans* A26 cells and incubated for 48 hours at 30°C. A 0.05 ml aliquot from the tube that contained the highest concentration of LY121019 that permitted growth was transferred to another series of tubes containing YNB broth with 2-fold dilutions of LY121019. A total of 17 serial transfers of *C. albicans* A26 were made in LY121019-containing medium, the 18th serial transfer was in LY121019 free medium.

### Determination of Fungicidal Activity

Inocula of $1\times10^5$ log phase cells of *C. albicans* A26 were added to tubes containing 5 ml YNB broth and concentrations of LY121019, amphotericin B, or nystatin ranging from 0.312~5.0 μg/ml. Following 48 hours incubation at 30°C, 0.1 ml aliquots were removed from each tube and plated on YNB agar plates. Fungicidal activity was determined as 99.9% killing of *C. albicans* A26 following 48-hour exposure to the antifungal agents.

### Effect of Antifungal Agents on Non-Metabolizing Cells of *C. albicans*

LY121019, miconazole, or amphotericin B was added to YNB broth in tubes at 40 μg/ml and diluted 2-fold to 0.078 μg/ml. $5\times10^5$ log phase cells of *C. albicans* A26 were added to 5 ml YNB broth containing antifungal agents. Inoculated tubes with or without antifungal agents were incubated at 4°C for 48 hours. The number of viable *C. albicans* A26 cells was determined from triplicate plate counts made on YNB agar after 48 hours incubation at 30°C.

### Scanning Electron Microscopy

YNB agar plates were prepared containing LY121019 at concentrations of 0.3, 0.75, 1.5 and 3 μg/ml and a control plate without antibiotic. Nuclepore filters were placed on the agar surface and the plates were prewarmed to 30°C. Each filter was inoculated with 10 μl of diluted culture to attain approximately $10^5$ cells per filter. After incubation for 1, 3 or 5 hours, filter specimens were removed and placed in filter holders, rinsed with water and then fixed with 3% glutaraldehyde in cacodylate buffer for 1 hour. The specimens were then rinsed with 2 ml of buffer, dehydrated in ethanol, critical point dried in a Bomar apparatus and coated with gold in an Edward vacuum evaporator[9].

### Determination of $ED_{50}$ for Antifungal Agents against *C. albicans*

The procedures used to determine the $ED_{50}$ of LY121019 and amphotericin B in compromised mice were described previously[4]. $CD_1$ (Charles River) male mice weighing 18~20 g were used. Ten mice were used for each concentration of antifungal agent tested. All antifungal agents were administered intraperitoneally at 0, 4 and 24 hours post-infection.

### Recovery of *C. albicans* from Kidney Homogenates of Mice Treated with Antifungal Agents

The procedures used for recovering *C. albicans* from kidney homogenates of treated and untreated mice 24 hours following the last treatment were reported previously[5]. $CD_1$ mice were infected intravenously with $1\times10^5$ *C. albicans* A26 cells. Plate counts were made on YNB agar following incubation for 48 hours at 30°C.

Effect of Antifungal Agents on *C. albicans* Infections in the Gastrointestinal Tract of Mice

The procedures for establishing a *Candida* infection in the gastrointestinal tract of $CD_1$ mice and subsequent treatment with antifungal agents were described previously[6].

Effect of Antifungal Agents against Superficial *C. albicans* Infections

The procedures for producing superficial *C. albicans* infections using albino guinea pigs (350~500 g) were essentially the same as those reported previously[7]. Guinea pigs were inoculated on abraded skin with 1.0 ml of a saline suspension containing $2 \times 10^7$ cells/ml of *C. albicans* SC5314. The skin was gently abraded with a blunted 20 gauge needle in an overlapping horizontal and vertical pattern in an area approximately 25×25 mm. The inoculum was then rubbed into the abraded area with a cotton swab. Topical treatment twice daily was started 5 days post-infection. Two lesions were induced on each of two guinea pigs for each antifungal agent and controls. Lesion score (0~4) was determined subjectively on the degree of scaling, crusting, and erythema of superficial lesions.

Effect of Antifungal Agents against Rat Vaginal Candidiasis

The procedures used for establishing rat vaginal candidiasis were essentially the same as those reported previously[8]. In our studies, ovariectomized Wistar rats (Charles River) weighing 170~200 g were treated subcutaneously for 7 days with 7.2 mg/kg of estradiol. Rats in estrus phase were inoculated intravaginally with $1 \times 10^7$ log phase cells of *C. albicans* SC9172. LY121019 was formulated in a modified vanishing cream base. Nystatin cream (Mycostatin, Squibb) and miconazole cream (Monostat, Johnson and Johnson) were used. Rats were treated intravaginally with approximately 0.1 ml of each cream formulation or placebo cream twice daily for 8 days. At 2 and 8 days post-treatment, the recovery of *C. albicans* was determined by plating triplicate 0.1 ml samples of a vaginal wash. Vaginal washes from rats held dorsal side down were obtained by introducing 0.05 ml of sterile saline into the vaginal cavity with a micropipet. The saline was aspirated several times to insure thorough washing of the vaginal cavity. The vaginal washes were removed from the vaginal cavity using a micropipet and diluted in 0.5 ml sterile saline before plating. Vaginal washes were obtained from 6 rats treated with each antifungal agent and the placebo, and from untreated controls. The plating procedure used was the same as that described for the recovery of *C. albicans* from kidney homogenates. The comparative effectiveness of each antifungal agent to the controls and each other was determined using the student "T" test.

Toxicology Evaluation

LY121019 was administered by intravenous infusion (25 mg/ml; 2.2 ml/minute) to groups of 2 male and 2 female beagle dogs at doses of 0, 10, 30 or 100 mg/kg/day, five days a week for three months. The vehicle consisted of 4.6 g $NaH_2PO_4$, 4.73 g $Na_2HPO_4$ and 4.8 g NaCl per liter of 33% v/v polyethylene glycol 300 in distilled water. The vehicle was adjusted to pH 6.8 before use. Animals were examined daily for physical and behavioral signs of toxicity. Body weights were measured weekly. Hematology (leukocyte, erythrocyte, platelet, and reticulocyte counts, hemoglobin, packed cell volume, leukocyte differential, prothrombin time, erythrocyte morphology, fibrinogen, activated partial thromboplastin time), serum biochemistry (glucose, urea nitrogen, creatinine, total bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase), and urinalysis (pH, specific gravity, protein, glucose, occult blood and bilirubin) parameters were determined before the study began and at 1, 2, 7, 8 and 13 weeks. Concentrations of LY121019 in serum were determined by microbiological assay using *Aspergillus montevidensis* A35137 as the test organism. All serum or aqueous humor and cerebral spinal fluid samples were diluted 1:1 with a solution of 50% methanol-50% pH 6.0 phosphate buffer. Petri plates (100 mm×20 mm) were prepared containing 10 ml of Biochem Agar No. 3 medium with 0.3 ml of a standardized inoculum of *A. montevidensis* A35137. The Biochem Agar No. 3 medium consisted of the following: $K_2HPO_4$ 0.69 g, $KH_2PO_4$ 0.45 g, yeast extract 2.5 g, glucose 10.0 g, agar (purified) 20.0 g, distilled water to make 1,000 ml.

The standardized spore suspension was adjusted to 0.6 O.D. at 590 nm. Tobramycin (10 $\mu$g/ml) was added to the medium to prevent bacterial contamination. Four 6 mm diameter agar wells were cut from each plate in a symmetrical arrangement. A total 0.03 ml of serum sample was added to 2

agar wells and 2 agar wells received a reference point from the standard curve for LY121019. The Petri plates were incubated at 30°C for 48 hours. The potency of each sample was determined from a standard curve based on the zones of inhibition measured in millimeters. The sensitivity of this microbiological assay was 0.15 μg/ml of LY121019. Blood samples for serum were obtained at the end of the infusion, and 1/2, 1, 2, 4 and 6 hours later on the first day of dosing and at the same intervals after 1, 2 and 3 months treatment. On the last day of the study, dogs were dosed and samples of cerebral spinal fluid and aqueous humor collected for analysis of LY121019 content. At necropsy, liver, kidney, heart, adrenals, spleen, ovaries, testes and thyroid were removed and weighed. Bone marrow was obtained from ribs and processed for determination of the ratios of cells in the myeloid: erythroid series; body orifices, external and internal organs and tissues examined grossly. Specimens of the following organs and tissues were fixed and processed for histopathologic evaluation: kidney, liver, heart, lung, spleen, thymus, lymph node, salivary gland, pancreas, stomach, duodenum, jejunum, ileum, colon, ovary, uterus, adrenal, thyroid, testis, prostate, skin, mammary gland, skeletal muscle, urinary bladder, cerebrum, cerebellum, brain stem, pituitary, rib bone, rib bone marrow, eye, gallbladder and injection site (cephalic vein).

## Results

### *In Vitro* Susceptibility of *C. albicans*

The MIC's for LY121019 and amphotericin B against 96 *C. albicans* isolates are shown in Table 1. The $MIC_{50}$ and $MIC_{90}$ for LY121019, 0.625 and 1.25 μg/ml, were identical to those for amphotericin B, however, the range of MIC values for amphotericin B was broader than for LY121019.

Table 1. Activity of LY121019 and amphotericin B against 96 isolates of *C. albicans*.

| Drug | MIC (μg/ml) | | |
|---|---|---|---|
| | Range | $MIC_{50}$ | $MIC_{90}$ |
| LY121019 | <0.078~2.5 | 0.625 | 1.25 |
| Amphotericin B | 0.156~25* | 0.625 | 1.25 |

* MIC of this polyene-resistant isolate for amphotericin B 25 μg/ml and 2.5 μg/ml for LY121019.

### Activity of Antifungal Agents on Different Growth Media Using the Agar and Broth Dilution Tests

Table 2 show that neither the test medium,

Table 2. Comparison of the MIC of LY121019 with other antifungal agents on two fungal growth media against five *C. albicans* isolates using the broth dilution and agar dilution tests.

| Drug | MIC distribution (μg/ml)* | | | |
|---|---|---|---|---|
| | Agar dilution | | Broth dilution | |
| | YNB | SAB | YNB | SAB |
| LY121019 | 4/0.625<br>1/0.312 | 5/0.625 | 2/1.25<br>3/0.625 | 3/0.625<br>1/1.25<br>1/0.312 |
| Amphotericin B | 5/0.625 | 3/0.625<br>2/0.312 | 1/0.625<br>3/0.312<br>1/0.156 | 2/1.25<br>1/0.625<br>2/0.312 |
| Nystatin | 3/2.5<br>2/1.25 | 4/2.5<br>1/1.25 | 4/2.5<br>1/1.25 | 4/2.5<br>1/1.25 |
| Miconazole | 4/5.0<br>1/0.078 | 2/5.0<br>3/2.5 | 5/10 | 4/5.0<br>1/2.5 |
| Clotrimazole | 5/5.0 | 3/5.0<br>2/0.156 | 2/20<br>2/10<br>1/5.0 | 1/20<br>2/2.5<br>2/1.25 |
| 5-Fluorocytosine | 3/0.156<br>2/0.078 | 3/20<br>2/10 | 3/0.078<br>2/0.039 | 3/20<br>1/10<br>1/5.0 |

* Number isolates/MIC

Fig. 2. Effect of LY121019, amphotericin B or miconazole on non-metabolizing cells of *C. albicans* A26.

○ LY121019, □ amphotericin B, △ miconazole.

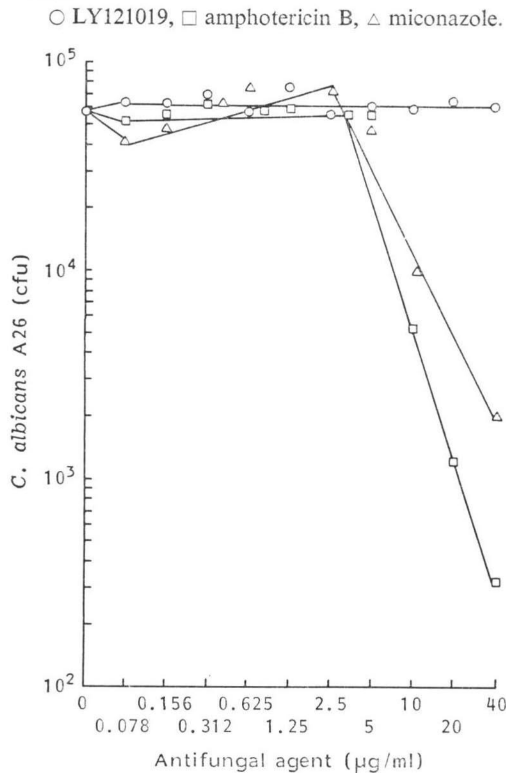



Table 3. Comparison of the *in vivo* anti-*Candida* activity of LY121019 with amphotericin B.

| Compound | $ED_{50}$ (95% confidence limits)* (mg/kg) |
|---|---|
| LY121019 | 7.4 (4.4～10.4) |
| Amphotericin B | 2.5 (0.1～5.1) |

* MIC vs. *C. albicans* A26: LY121019 0.625 μg/ml, amphotericin B 0.156 μg/ml. ×3 ip. Compromised mice (400 rad X-irradiation 24 hours preinfection).

YNB *versus* Sabouraud (SAB) nor the method of testing, agar dilution *versus* broth dilution, greatly affected the activities of LY121019, amphotericin B, or nystatin against 5 isolates of *C. albicans*. In contrast, the activities of miconazole, clotrimazole and especially 5-fluorocytosine were affected by both medium and test procedure.

### Fungicidal Activity

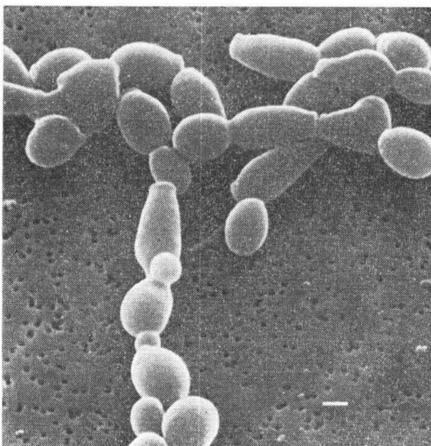
LY121019, amphotericin B and nystatin were fungicidal against *C. albicans* A26 at concentrations of 0.312, 0.625 and 1.25 μg/ml, respectively. Thus, the minimal fungicidal concentrations and MIC's of these agents for this isolate were identical.

### *In Vitro* Resistance Development

The MIC of LY121019 for *C. albicans* A26 increased gradually from 0.625 μg/ml to 5.0 μg/ml after 17 serial transfers in medium containing this agent. Following the 18th transfer in LY121019-free medium, the MIC was 2.5 μg/ml.

Fig. 3. Growth of *C. albicans* A26 following 3 hours at 30°C on nuclepore filters placed on an agar surface. Marker=1 μm

(A) Cells not exposed to LY121019.



(B) Cells exposed to 1.5 μg/ml LY121019.

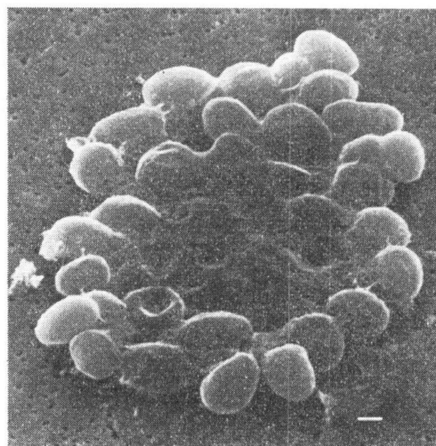


Table 4. Recovery of *C. albicans* from kidney homogenates of mice treated intraperitoneally with (A) LY121019 or amphotericin B commencing 24 hours post-infection then daily for 5 days or (B) LY121019 treated at 4 hours preinfection, at time of infection and then daily for 5 days.

| Antifungal antibiotic | Dose (mg/kg) | Recovery of *C. albicans* cfu/g kidney ± SE |
|---|---|---|
| A. Treatment 24 hours after infection then daily for 5 days | | |
| LY121019 | 50 | $2.4\times10\pm0.6$ |
| Amphotericin B | 50 | $2.9\times10\pm0.3$ |
| Controls | 0 | $8.7\times10^4\pm1.4$ |
| B. Treatment at 4 hours preinfection, at infection, then daily for 5 days | | |
| LY121019 | 25 | $<1\times10$ |
| | 12.5 | $<1\times10$ |
| | 6.25 | $4.2\times10^2\pm1.4$ |
| Controls | 0 | $1\times10^4\pm3.0$ |

### Activity against Non-metabolizing *C. albicans* Cells

Exposure of *C. albicans* A26 to 40 μg LY121019/ml for 48 hours at 4°C did not affect viability (Fig. 2). Exposure to similar concentrations of amphotericin B or miconazole effected decreases in viable cells of more than 2-logs and 1-log, respectively.

### Scanning Electron Microscopic Study of Antifungal Action

When grown on a filter placed on the YNB medium, *C. albicans* A26, formed microcolonies of growing spherical and tubular shaped cells. These yeast cells formed a chain of interconnected or closely associated cells due to the budding process. The cell surfaces were generally smooth and featureless except for the button-like scar from the budding process (Fig. 3A). The morphology of cells treated with LY121019 at 0.75 μg/ml or higher show changes as in Fig. 3B, treated with 1.5 μg/ml for 3 hours. No budding processes were observed and the center of the microcolony was covered with extracellular debris. The visible cells showed stringy attachments to other cells and small vacuoles on the cell surfaces. Some *C. albicans* A26 cells were partially collapsed.

### $ED_{50}$ Determinations

The $ED_{50}$ of LY121019 for experimental systemic *C. albicans* infections in compromised mice shown in Table 3 was approximately 3-fold higher than the $ED_{50}$ for amphotericin B.

### Recovery of *C. albicans* from Infected Mouse Kidneys

Administration of 50 mg/kg doses of LY121019 or amphotericin B commencing 24 hours post-infection then daily for 5 days resulted in a greater than 3-log reduction in the numbers of *C. albicans* in kidney homogenates of systemically infected mice (Table 4). When LY121019 administered at 4 hours preinfection, at the time of infection, and then daily for 5 days, doses of 12.5 and 25 mg/kg effected in a greater than 3-log reduction in numbers of *C. albicans* in kidney homogenates. Doses of 6.25 mg/kg LY121019 effected a 2-log reduction in the numbers of *C. albicans*.

### Recovery of *C. albicans* from the Gastrointestinal Tract of Infected Mice

The activity of orally administered LY121019 or nystatin against *C. albicans* infections in the gastrointestinal tract of mice is summarized in Table 5. Doses of 50 and 100 mg/kg LY121019 effected

Table 5. Activity of LY121019 and nystatin administered orally against *C. albicans* infections in the gastrointestinal tract of mice.

| Compound (mg/kg) | | Recovery of *C. albicans* cfu/g feces $\pm$SE (days post treatment) | | | | |
|---|---|---|---|---|---|---|
| | | Day 0 | Day 2 | Day 6 | Day 8 | Day 10 |
| LY121019 | 50 | $6.4\times10^3\pm1.8$ | $1.8\times10^3\pm0.75$ | $<10$ | $<10$ | $<10$ |
| | 100 | $3.7\times10^4\pm1.6$ | $3.3\times10\pm3.3$ | $<10$ | $<10$ | $<10$ |
| Nystatin | 50 | $1.0\times10^3\pm0$ | $2.2\times10^4\pm0.1$ | $3.7\times10^4\pm0.38$ | $<10$ | $<10$ |
| | 100 | $5.3\times10^3\pm3.3$ | $5.1\times10^3\pm1.4$ | $<10$ | $<10$ | $<10$ |
| Untreated controls | | $8.7\times10^3\pm2.3$ | $5.4\times10^5\pm0.05$ | $9.0\times10^4\pm4.0$ | $1.0\times10^5\pm0$ | $2.7\times10^5\pm7.0$ |

Table 6. Evaluation of 5% LY121019 and 3% nystatin in a superficial candidiasis guinea pig model.

| Treatment | Lesion | Days post-treatment/average lesion score | | | |
|---|---|---|---|---|---|
| | | 5 | 6 | 7 | 8 |
| 5% LY121019 cream | Treated | 1.25 | 0.5 | 0 | 0 |
| | Placebo | 2.75 | 3.0 | 3.0 | 3.0 |
| | Control | 3.75 | 4.0 | 4.0 | 4.0 |
| 3% Nystatin cream | Treated | 1.75 | 1.0 | 0 | 0 |
| | Placebo | 3.5 | 3.0 | 3.0 | 3.0 |
| | Control | 3.5 | 4.0 | 3.5 | 3.5 |

MIC vs. *C. albicans* SC5314: LY121019 0.625 μg/ml, nystatin 5.0 μg/ml.

a greater than 4-log reduction in the numbers of *C. albicans* at 6 days post-treatment. With doses of 50 mg/kg nystatin this endpoint was reached on the 8 post-treatment day. Doses of 100 mg/kg effected a greater than 4-log reduction in the numbers of *C. albicans* compared to the untreated controls at 6 days post-treatment.

## Activity against Superficial *C. albicans* Infections

The efficacy of 5% LY121019 or 3% nystatin cream formulations against superficial *C. albicans* infections on guinea pigs is summarized in Table 6. Lesion scores of LY121019 or nystatin treated animals compared with placebo and untreated, were reduced after 5 daily topical treatments. At 7 days post-treatment, superficial lesions on animals treated with either antifungal agent were cured. In contrast, placebo-treated and untreated control animals showed little reduction in the scaling, crusting or erythema of cutaneous lesions.

## Activity against Vaginal *C. albicans* Infections

The comparative efficacies of 3% LY121019, 2% miconazole and 3% nystatin cream formulations against rat vaginal infections are presented in Table 7. At 2 days post-treatment, local treatments of 3% LY121019, 2% miconazole or 3% nystatin resulted in a significant reduction ($P<0.05$) in the recovery of *C. albicans* from vaginal washes compared with placebo treated or untreated controls. There was a significant difference ($P<0.05$) in the recovery of *C. albicans* between 2% miconazole and 3% nystatin, but not between 3% LY121019 and 3% nystatin or 2% miconazole. After 8 days of local treatments, the recovery of *C. albicans*, compared with placebo treated or untreated controls, was not significantly different between 3% LY121019 and 2% miconazole. However, the recovery of *C. albicans* with 3% LY121019 treatments and 2% miconazole treatments were significantly different ($P<0.05$) from the recovery obtained with 3% nystatin treatments. The pre- and post-treatment MIC's

Table 7. Recovery of *C. albicans* from vaginal washes of rats inoculated intravaginally and treated locally with LY121019, miconazole or nystatin.

| Antifungal agent | Recovery of *C. albicans** cfu/ml±SE | |
|---|---|---|
| | 2 days post-treatment | 8 days post-treatment |
| 3% LY121019 | $2.5\times10^2\pm2.0$ | $3.2\times10^3\pm1.6$ |
| 2% Miconazole | $2.0\times10^3\pm4.0$ | $8.0\times10^2\pm2.5$ |
| 3% Nystatin | $6.3\times10\pm1.6$ | $6.3\times10\pm2.0$ |
| Placebo controls | $2.5\times10^5\pm1.6$ | $2.0\times10^4\pm2.5$ |
| Untreated controls | $8.0\times10^4\pm2.0$ | $4.0\times10^4\pm2.0$ |

* MIC (μg/ml) for *C. albicans* SC9172: LY121019 0.312 (before treatment), 0.625 (after treatment); miconazole 5.0, 5.0; nystatin 2.5, 5.0.

Table 8. Effect on body weight gain and relative liver weight in dogs administered LY121019 intravenously for three months.

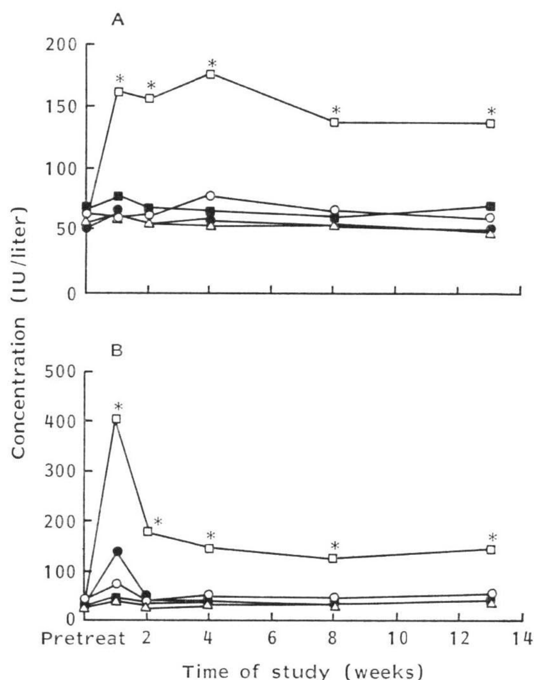
| Dose group | Mean body weight gain (% of initial weight) | Relative liver weight (g/100 g body weight) |
|---|---|---|
| Control | −1.8 | 2.68±0.06[a] |
| Vehicle | −3.0 | 2.85±0.19 |
| 10 mg/kg/day | −3.5 | 2.76±0.09 |
| 30 mg/kg/day | −2.5 | 3.09±0.16 |
| 100 mg/kg/day | −8.2 | 4.28±0.04[b] |

[a] Mean±SEM.
[b] Significantly different from control DUNNETT's t, $P<0.05$.

Fig. 4. Effect of LY121019 on serum alkaline phosphatase (A) and alanine transaminase (B).

○ Control, △ vehicle, ● 10 mg/kg/day, □ 30 mg/kg/day, □ 100 mg/kg/day.

* Statistical significance DUNNETT's t at $P<0.05$.




for the three antifungal agents against *C. albicans* were not greater than 2-fold.

## Evaluation for Toxicity

Outward manifestations of toxicity were encountered only in dogs given 100 mg LY121019/kg. As indicated in Table 8, these dogs showed a 5% weight loss and exhibited vasodilation, swelling and edema around the face and mouth, itching, a decrease in blood pressure, and tender, swollen and flaccid veins. All of the above effects except for the effects on veins, were reversed one-half to three hours after dosing. The clinical signs of toxicity were observed in some animals on the initial day of the study, occurred with variable severity in a given animal, and generally did not increase in severity as the study progressed.

Administration of LY121019 did not affect the formed elements of peripheral blood nor did it modify either physical or morphologic characteristics of urine. At doses of 100 mg/kg, but not at lower doses, LY121019 effected significant elevations of alkaline phosphatase and alanine transaminase at 1, 2, 4, 8 and 13 weeks (Fig. 4). Other clinical chemistry parameters were not affected by this or lower doses of LY121019.

The concentrations of LY121019 in serum were directly related to the dose administered (Fig. 5). Concentrations at the end of the infusion ranged from 112~156, 344~622 and 558~1,064 μg/ml for dogs receiving daily doses of 10, 30 and 100 mg/kg, respectively. LY121019 was present in serum 6 hours in dogs that received 30 and 100 mg/kg doses. There was no evidence of accumulation or altered peak serum concentrations. Analysis of cerebral spinal fluid and aqueous humor for LY121019 1

Fig. 5. Serum concentrations of LY121019 after intravenous infusion to dogs.

○ 10 mg/kg/day, △ 30 mg/kg/day, □ 100 mg/kg/day, zero time equivalent to immediately after infusion completed.

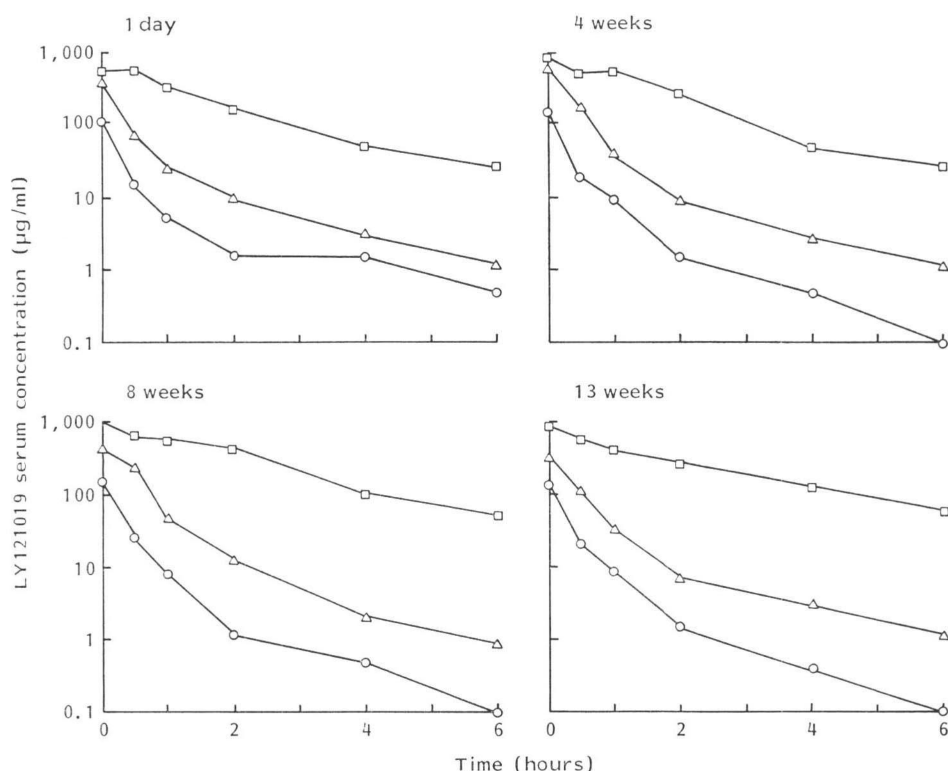



Table 9. Summary of pathologic diagnoses related to the intravenous administration of LY121019 to dogs for three months.

| | | Control | | Vehicle | | 10 mg/kg | | 30 mg/kg | | 100 mg/kg | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M* | F | M | F | M | F | M | F | M | F |
| Injection site | Acute inflammation | | | | 1 | | | | | 2 | 2 |
| | Subacute inflammation | | | | | | | | 1 | | |
| | Chronic inflammation | | | | | | 1 | | 1 | | 1 |
| | Hemorrhage | | | | | | 1 | 1 | 2 | 2 | 2 |
| | Focal phlebitis | | | | | | | | 1 | 2 | |
| | Thrombosis | | | | | | | | | | 1 |
| Liver | Mild centrilobular fatty vacuolation | | | | | | | 2 | 2 | | |
| | Marked centrilobular fatty vacuolation | | | | | | | | | 2 | 2 |

* Two animals used. M male, F female.

hour after dosing indicated that LY121019 was not present in these fluids.

At necropsy, no LY121019-related effects on bone marrow were observed. As shown in Table 8, analysis of organ weight data indicated an effect on liver weight. Relative liver weight was significantly increased for dogs from the 100 mg/kg group. LY121019-related lesions observed in the liver and at the site of injection are shown in Table 9. A dose-related hemorrhage at the injection site was pre-

sent only in dogs given LY121019. Inflammation occurred in some but not all dogs from each injected group including the vehicle control. More instances of inflammation were observed in dogs given 100 mg/kg than in other groups. Phlebitis of a focal nature occurred in the cephalic vein (site of injection) of one dog given 30 mg/kg/day and in two dogs given 100 mg/kg/day. Centrilobular fatty change was observed in hepatocytes from all dogs given 30 or 100 mg/kg. The change was mild and tended to be diffuse throughout the lobules in the 30 mg/kg dogs and marked in the 100 mg/kg dogs.

## Discussion

LY121019 showed potent *in vitro* activity that compared favorably with amphotericin B against a large number of *C. albicans* isolates. Furthermore, MIC values for LY121019 showed little variation when determined by the agar or broth dilution test using YNB or Sabouraud medium. In contrast to 5-fluorocytosine, the antifungal activity of LY121019 was unaffected by the nutrients in Sabouraud medium. Other properties of the *in vitro* antifungal activity include a fungicidal action at concentrations required for the MIC. Our studies indicate that LY121019 has a low potential for resistance development since the MIC for *C. albicans* increased only gradually 8-fold during 17 serial transfers in the presence of LY121019. The final transfer of *C. albicans* into LY121019-free medium showed only a 2-fold decrease in the MIC after exposure to LY121019. Whether or not this low level of resistance is stable remains to be determined.

Non-metabolizing cells of *C. albicans* were not inhibited by LY121019 whereas miconazole and amphotericin B showed increased MIC's against non-metabolizing cells at 4°C compared with MIC's obtained against normal metabolizing cells at 30°C. Because the mode of action of amphotericin B and miconazole are different from that of LY121019, this could account for the differential antifungal action of these agents. The mode of action of polyenes such as amphotericin B involves their irreversible binding to sterol components in cell membranes resulting in destruction of the membrane integrity[10]. In contrast, imidazoles such as miconazole inhibit the biosynthesis of sterol components of cell membranes resulting in cell leakage[11]. We have shown that LY121019 causes severe damage to the *C. albicans* cell wall. Echinocandin B and aculeacin are polypeptide antifungal antibiotics related to LY121019 that have been reported to inhibit the incorporation of glucan into the *C. albicans* cell wall[12,13]. The antifungal action of LY121019 requires actively metabolizing *C. albicans* cells, whereas the antifungal activity of polyene and imidazole antifungal agents against non-metabolizing cells is only decreased.

The *in vitro* anti-*Candida* activity of LY121019 correlated with *in vivo* efficacy. The $ED_{50}$ obtained for LY121019 in the treatment of experimental systemic *C. albicans* mouse infections compared favorably with the $ED_{50}$ obtained for amphotericin B. LY121019 was highly effective at doses of 12.5 and 25 mg/kg in reducing the recovery of *C. albicans* from kidneys. LY121019 administered orally was at least as effective as nystatin in eradicating *C. albicans* from the gastrointestinal tract of infected mice. *Candida* overgrowth in the gastrointestinal tract is known to be a predisposing factor of vaginal or systemic candidiasis. Agents that safely and effectively eliminate *Candida* colonized in the gastrointestinal tract would be useful in preventing the development of *Candida* infections. Topical application of LY121019 was as effective as nystatin against superficial *C. albicans* infections on guinea pigs. Further *in vivo* studies of the topical antifungal activity of LY121019 are needed to determine the optimum formulation and treatment regimen. There is a continuing need for antifungal agents that are effective against *Candida* infections of the skin, nails and especially chronic mucocutaneous candidiasis[14]. LY121019 administered intravaginally showed effectiveness comparable to clinically used agents such as nystatin and miconazole against rat vaginal candidiasis. Although vaginal candidiasis in many cases can be treated effectively by antifungal agents, this *Candida* infection can be intractable, recurrent, and become a distressing disease[15]. Clearly, more effective agents are needed for vaginal candidiasis.

The results of the toxicologic evaluation demonstrate that LY121019 can be safely given intravenously to dogs. Doses of 10, 30 or 100 mg/kg/day, five days per week for 13 weeks did not result in lethality or severe signs of toxicity. The serum concentrations of LY121019 detected during this study

indicated there was no accumulative toxicity. The absolute no-effect level was 10 mg/kg/day. Toxicity observed included hepatotoxicity as indicated by increases in alkaline phosphatase and alanine transaminase and histologic evidence of centrilobular fatty vacuolation; inflammation, hemorrhage, phlebitis and thrombosis at the injection site; and clinical signs of vasodilation, swelling around the face and mouth, edema, itching, and an apparent decrease in blood pressure. The clinical signs were thought to be indicative of an increase in circulating histamine levels. Subsequent preliminary experiments in the rat with intraperitoneal doses of LY121019 demonstrated that LY121019 can increase serum histamine levels. The hepatotoxicity observed was not accompanied by evidence of necrosis and, therefore, would have to be considered potentially reversible upon discontinuing LY121019 treatment.

The toxicity data for LY121019 compare very favorably with other studies on the toxicity of amphotericin B in dogs that showed doses of 2.5 mg/kg were not tolerated and doses of 1.25 mg/kg produced severe nephrotoxicity[18].

LY121019 is a novel antifungal antibiotic that possesses potent *in vitro* activity against *C. albicans*. Furthermore, LY121019 showed significant effectiveness in experimental animal infections that are representative of a number of clinically important *C. albicans* infections. LY121019 was also well-tolerated at doses required for *in vivo* efficacy in these experimental *C. albicans* infections. In addition, toxicity studies in dogs indicate that LY121019 is at least 20-fold less toxic than amphotericin B.

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