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# New Class of Antifungal Agents: Jasplakinolide, a Cyclodepsipeptide from the Marine Sponge, *Jaspis* Species

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**Jasplakinolide is a cyclodepsipeptide which represents a new class of antifungal agents with potent activity against *Candida albicans*. Jasplakinolide is fungicidal against *C. albicans* with both a MIC and a minimum lethal concentration of 25 μg/ml in a broth dilution assay. This activity compares to that of the imidazole miconazole nitrate, which had a MIC of 6.2 μg/ml and a minimum lethal concentration of 50 μg/ml in the same assay. Topical administration of 2% jasplakinolide cream against a murine vaginal *C. albicans* infection was equivalent in efficacy to administration of miconazole nitrate at 2%. Subcutaneous administration of jasplakinolide was not effective against a systemic murine *C. albicans* infection.**

Imidazole antifungal agents, such as clotrimazole and miconazole nitrate, are widely used as topical agents against *Candida* species. The mechanism of action is primarily fungistatic, with fungicidal activity only at high concentrations and with increased incubation time (9, 10). We describe here the in vitro and in vivo activity of jasplakinolide, a novel compound of a new class with fungicidal activity. It is a cyclodepsipeptide composed of three amino acids and an oxytrimethylnonanoyl group, isolated from the soft-bodied sponge *Jaspis* species (order, Astrophorida; family, Jaspidae) collected off the shore of the island of Benga, Fiji (3, 13). It was purified by Phillip Crews, University of California, Santa Cruz (Fig. 1).

## MATERIALS AND METHODS

**In vitro activity.** The MIC of jasplakinolide was determined by means of a broth dilution assay (6, 7). Jasplakinolide and miconazole nitrate were dissolved in dimethyl sulfoxide, and their concentrations were adjusted with Sabouraud dextrose broth (Difco Laboratories, Detroit, Mich.). The drugs were serially diluted twofold in Sabouraud dextrose broth in a 96-well, round-bottom microdilution plate (Linbro Division, Flow Laboratories, Hamden, Conn.). Final drug concentrations ranged from 200 to 0.2 μg/ml. The maximum solvent concentration was 2%. The inoculum size was approximately $5.3 \times 10^4$ *Candida albicans* cells per ml. MICs were determined after 18 h of incubation at 37°C.

A disk assay was done to screen for activity against *Staphylococcus aureus* ATCC 6538P, *Streptococcus pyogenes* ATCC 8668, *Escherichia coli* Smith, *Pseudomonas aeruginosa* ATCC 10145, and clinical isolates of *Mycoplasma* species, *C. albicans*, and *Trichophyton mentagrophytes*. Bacteria were tested on tryptic soy agar by using a Kirby-Bauer procedure (1). *Mycoplasma* isolates were similarily tested by using *Mycoplasma* agar. The fungi were seeded in a modified Sabouraud agar (10% dextrose) at 10,000 PFU/ml. Disks (diameter, 0.5 in. [1.27 cm]) containing 100 μg of jasplakinolide were placed on the agar surface. Commercial gentamicin disks containing 10 μg of drug were used as controls for the bacteria and *Mycoplasma* isolates. Disks containing 100 μg of miconazole nitrate were used as the fungal control.

The minimal lethal concentration (MLC) was determined by transferring the contents of a well (ca. 200 μl) in the microdilution plate used to determine the MIC to a tube containing 5 ml of Sabouraud dextrose broth. The tubes were incubated for 2 days at 37°C. The lowest concentration of drug at which no growth was observed was determined to be the MLC.

The MICs of the drugs against different isolates of several *Candida* species were determined by using an agar dilution procedure with a modified Sabouraud agar (1% dextrose) (11). Final drug concentrations ranged from 100 to 0.3 μg/ml in half-log increments. Plates were incubated overnight at 37°C, and the lowest drug concentration at which no growth was observed was the MIC.

**Jasplakinolide uptake.** *Candida* species were grown up overnight in Sabouraud dextrose broth and counted, and $3 \times 10^8$ cells per tube were dispensed into culture tubes, each containing 0.75 ml of broth. Jasplakinolide was dissolved in 50% dimethyl sulfoxide, and 3 μl was added to each culture tube to yield a final concentration of 20 μg/ml. Samples were incubated at 37°C for 2, 6, or 18 h; washed three times each with 12 ml of saline; and frozen until analyzed. These time points were selected because *Candida* species do not grow for the first 2 h under the described test conditions; therefore, any differences observed at earlier time points could not be correlated with a biologically meaningful effect.

For analysis of jasplakinolide content, the cell samples were sonicated in 0.2 ml of 50% acetonitrile and centrifuged to remove particulate material. A 50-μl portion of each sample was injected into a high-pressure liquid chromatography apparatus (LDC/Milton Roy, Riviera Beach, Fla.) with a Partisil 5 ODS-3 reversed-phase $C_{18}$ column (0.46 by 10 cm; Whatman, Inc., Clifton, N.J.). The mobile phase was 50 mM potassium phosphate (pH 4)-acetonitrile at 1:1 (vol/vol). The $A_{280}$ was monitored, and peaks were identified, integrated, and quantified by comparison with a jasplakinolide standard.

**Macromolecule incorporation experiments.** *Candida* isolates were incubated overnight, counted, and dispensed into culture tubes at $1.5 \times 10^7$ cells per 15 ml. After the tubes had been incubated for 3 h at 37°C, jasplakinolide was added to alternate tubes to yield a final concentration of 25 μg/ml. The optical density was measured at 650 nm every 30 min to determine the growth rate. At intervals following jasplakinolide addition, 100-μl samples were transferred to tubes

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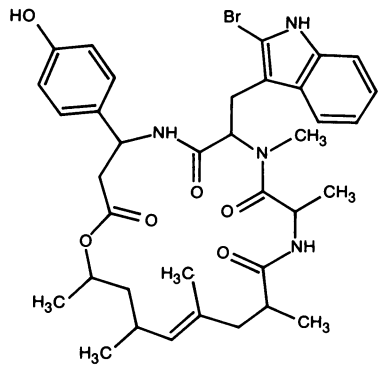

FIG. 1. Molecular structure of jasplakinolide.

containing 5 μCi of either [*methyl*-$^3$H]thymidine, [5-$^3$H]uridine, or a $^3$H-amino acid mixture. After incubation for 15 min with the radiolabels, 100% trichloroacetic acid was added to each tube to yield a final concentration of 20% trichloroacetic acid. Samples were then filtered through GF/F glass fiber filters (Whatman) with three 5-ml washes of 5% trichloroacetic acid–20 mM sodium $PP_i$ and one wash of 5 ml of methanol. The bound tritium content of the filters was measured by liquid scintillation.

**In vivo activity.** The in vivo toxicity of jasplakinolide was tested by subcutaneous or peroral administration to female Swiss-Webster or ICR mice. Mice were given various levels of jasplakinolide from 8 mg/kg of body weight down to 0.03 mg/kg in single or multiple doses. Multiple doses were given twice daily with a 6-h interval for a maximum of 3 days. The mice were observed for effects such as ruffled appearance, edema, weight loss, lowered body temperature, tissue necrosis, morbidity, and mortality.

In vivo efficacy was determined by topical application of jasplakinolide against a vaginal *C. albicans* infection and parental administration against a systemic *C. albicans* infection. The topical activity was determined by using female Swiss-Webster mice (Simonsen Laboratories, Gilroy, Calif.) (12). Estrus was induced by subcutaneous injection of β-estradiol (Sigma Chemical Co., St. Louis, Mo.) at 50 μg per mouse on days 7, 5, and 3 preinfection. A maintenance dose was given on day 4 postinfection. The mice were infected vaginally (ca. 0.07 ml per mouse) via a 20-gauge intubation needle with a saline suspension of *C. albicans* which contained $1.18 \times 10^7$ organisms per ml.

Jasplakinolide and miconazole nitrate were each tested at 2% in a water-oil emulsion. Treatments were administered vaginally by using a syringe with an 18-gauge intubation needle at 6, 24, 48, and 72 h postinfection. A vaginal wash (0.2 ml), which contained 100 μg of oxytetracycline (Liquamicin; Pfizer Inc., New York, N.Y.) per ml and 20 μg of gentamicin (Garamycin; Schering Pharmaceutical Corp., Kenilworth, N.J.) per ml to reduce bacterial contamination, was performed for each mouse on days 1 and 4 posttreatment (days 4 and 7 postinfection). Each wash was transferred to a Nickerson (BiGGY) agar (Difco) plate and spread over the entire agar surface with a bent glass rod. The plates were incubated at 37°C for 2 to 3 days. Plates containing more than 10 *Candida* colonies were considered to be positive. The data were analyzed by using Fisher exact probability statistics (8).

The subcutaneous activity of jasplakinolide against a systemic *C. albicans* infection in mice was determined as previously described (4). In brief, female ICR-SPF mice (average weight, 20 g) were challenged intravenously with $4.5 \times 10^6$ cells of a human clinical isolate of *C. albicans*. Jasplakinolide was given subcutaneously at 24, 30, 48, 53, 72, and 78 h after the challenge. Ketoconazole (50 mg/kg of body weight) was administered perorally at 24, 28, 48, and 72 h after challenge. Mortality was recorded daily for 10 to 11 days. The data were analyzed by using Mann Whitney U statistics (5).

TABLE 1. Agar dilution MICs for isolates

| Test agent | Organism (no. of isolates) | MIC (μg/ml) for individual isolates ($ED_{50}$)[a] |
|---|---|---|
| Jasplakinolide | *C. albicans* (15) | ≤0.3–3 (1) |
| | *C. tropicalis* (6) | ≥100 |
| | *C. glabrata* (6) | ≤0.3–3 (1) |
| | *C. parapsilosis* (2) | 1 |
| | *C. pseudotropicalis* (1) | ≤0.3 |
| | *Pityrosporum pachydermatis* (1) | >100 |
| Clotrimazole | *C. albicans* (15) | 3 |
| | *C. tropicalis* (6) | ≤0.3–3 (3) |
| | *C. glabrata* (6) | ≤0.3 |
| | *C. parapsilosis* (2) | ≤0.3 |
| | *C. pseudotropicalis* (1) | 1 |
| | *Pityrosporum pachydermatis* (1) | ≤0.3 |
| Miconazole nitrate | *C. albicans* (15) | 10 |
| | *C. tropicalis* (6) | 1–100 (10) |
| | *C. glabrata* (6) | ≤0.3 |
| | *C. parapsilosis* (2) | ≤0.3 |
| | *C. pseudotropicalis* (1) | 1 |
| | *Pityrosporum pachydermatis* (1) | ≤0.3 |

[a] $ED_{50}$, 50% Effective dose; dose (in micrograms per milliliter) at which 50% of isolates were inhibited.

## RESULTS

In vitro, the broth dilution MIC of jasplakinolide was 25 μg/ml, whereas that of miconazole nitrate was 6.2 μg/ml. However, the MLC of jasplakinolide was also 25 μg/ml, whereas the MLC of miconazole nitrate was >50 μg/ml. In a disk diffusion assay, jasplakinolide was not active against *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *Mycoplasma* species, or *T. mentagrophytes*. It was active only against *C. albicans*. The gentamicin disk was active against the bacteria and *Mycoplasma* species. Miconazole nitrate was active against the fungi.

The in vitro activity of jasplakinolide against several *Candida* strains was similar to those of clotrimazole and miconazole nitrate (Table 1). Jasplakinolide, however, lacked activity against *C. tropicalis*. There was no significant difference in uptake of jasplakinolide among the *Candida* strains that might account for the lack of activity against *C. tropicalis* (Table 2).

The basal rate of [$^3$H]thymidine incorporation was considerably higher in *C. albicans* than in *C. tropicalis* (Fig. 2). In this experiment, untreated organisms maintained a doubling time of 75 min for the first 2 h following treatment and then gradually slowed to a doubling time of 160 min at the end of the experiment (data not shown). Treated samples divided about 30% more slowly, but followed the same pattern as the untreated controls. Jasplakinolide had a reduced effect on cell division owing to the high inoculum used; this inoculum dependency was observed consistently. Treatment with jasplakinolide drastically reduced the rate of [$^3$H]thymidine

TABLE 2. Uptake of jasplakinolide by *Candida* species

| *Candida* species | Length of incubation (h) | Amt of jasplakinolide per $10^6$ cells (ng) |
|---|---|---|
| *C. albicans* | 2 | 1.16 |
| | 6 | 1.14 |
| | 18 | 1.37 |
| *C. glabrata* | 2 | 1.12 |
| | 6 | 1.17 |
| | 18 | 2.17 |
| *C. parapsilosis* | 2 | 0.96 |
| | 6 | 1.10 |
| | 18 | 1.64 |
| *C. pseudotropicalis* | 2 | 1.42 |
| | 6 | 1.77 |
| | 18 | 2.04 |
| *C. tropicalis* | 2 | 1.10 |
| | 6 | 1.26 |
| | 18 | 1.60 |

incorporation by *C. albicans*, particularly during the most rapid period of cell division, but had little effect on [$^3$H]thymidine incorporation by *C. tropicalis*. Later in the incubation, when *C. albicans* growth and basal [$^3$H]thymidine incorporation slowed, jasplakinolide had little effect on [$^3$H]thymidine incorporation by that species. In both species, jasplakinolide had a relatively minor effect on incorporation of [$^3$H]uridine and $^3$H-amino acids. Thus, the major observable effect of jasplakinolide in this experimental model was to suppress *C. albicans* DNA synthesis during the log phase. We did not determine whether this was due to a direct effect of the drug upon DNA replication or to some secondary mechanism which was reflected here by a suppression of DNA synthesis.

Mortal toxicity was seen with as little as 8 mg/kg of body weight when jasplakinolide was given as a single oral dose or 4 mg/kg when it was administered subcutaneously. It was also lethal when given to ICR mice in two 2-mg/kg doses with a 6-h interval. A single 2-mg/kg treatment was not lethal. Necrosis of tissue was seen at the site of injection in mice treated with 1, 0.3, and 0.1 mg/kg.

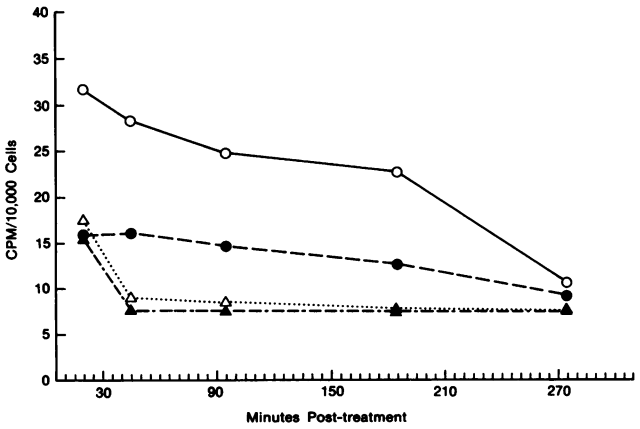


FIG. 2. Incorporation of [5-$^3$H]thymidine into *Candida* species. Symbols: ○, *C. albicans* (untreated); ●, *C. albicans* (jasplakinolide treated); △, *C. tropicalis* (untreated); ▲, *C. tropicalis* (jasplakinolide treated).

TABLE 3. In vivo topical activity of 2% formulations against a vaginal *C. albicans* infection

| Treatment | % Negative cultures[a] on: | |
|---|---|---|
| | Day 4 postinfection | Day 7 postinfection |
| Jasplakinolide | 53 | 47 |
| Miconazole nitrate | 47 | 47 |
| Placebo | 7 | 7 |
| None | 7 | 7 |

[a] There were 15 mice per group.

The activity of topically administered jasplakinolide against a murine vaginal *C. albicans* infection was equivalent to that of miconazole nitrate (Table 3) (8). Treatment with jasplakinolide subcutaneously at 0.1 or 0.03 mg/kg was not effective against a systemic *C. albicans* infection. Treatment with ketoconazole at 50 mg/kg was statistically more effective than treatment with jasplakinolide at 0.03 mg/kg ($P < 0.01$), but was not more effective than treatment with jasplakinolide at 0.1 mg/kg. Treatment with jasplakinolide at 1 or 0.3 mg/kg accelerated mortality due to toxicity (Fig. 3).

## DISCUSSION

Jasplakinolide represents a new class of antifungal agent that is novel both structurally and functionally. A unique attribute of this compound is its ability to kill the fungus at the same concentration as that required to inhibit growth. This is a departure from the activity seen with the imidazole antifungal agents, which are fungicidal only at high concentrations and after prolonged incubation (9, 10). The in vitro activity of jasplakinolide is good against all the *Candida* strains tested except *C. tropicalis* (Table 1), which is resistant to its action. Uptake of jasplakinolide by *C. tropicalis* was examined and is not a factor in the resistance of this organism. The results of the incorporation experiments, however, suggest that jasplakinolide inhibits DNA synthesis with greater potency in *C. albicans* than in *C. tropicalis*.

Topical administration of 2% jasplakinolide is very effective in reducing infection. Treatment with jasplakinolide at

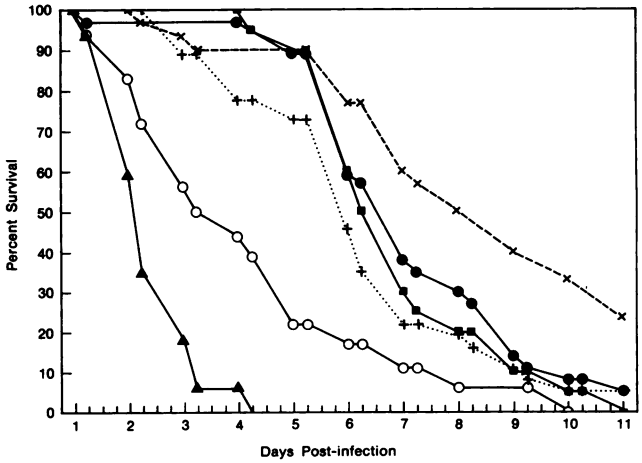


FIG. 3. Effect on a systemic murine *C. albicans* infection of subcutaneous treatment with jasplakinolide at 1, 0.3, 0.1, or 0.03 mg/kg of body weight twice daily or ketoconazole administered perorally once daily at 50 mg/kg for 3 days. Symbols: ▲, 1 mg/kg; ○, 0.3 mg/kg; ●, 0.1 mg/kg; ■, 0.03 mg/kg; ×, ketoconazole; +, untreated control.

subtoxic levels is ineffective against the systemic infection. Treatment twice daily for 3 days at 1 or 0.3 mg/kg in a systemic *Candida* infection accelerated mortality. Uninfected mice treated likewise showed no signs of toxicity. The toxicity and lack of broad-spectrum activity of jasplakinolide limit its development. However, the results of tests of jasplakinolide and other newly discovered cyclic depsipeptides with activity against *C. albicans* may provide a new direction for antifungal research (2).

## ACKNOWLEDGMENTS

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