ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1990, p. 587–593
0066-4804/90/040587-07$02.00/0

Vol. 34, No. 4

Evaluation of Nikkomycins X and Z in Murine Models of Coccidioidomycosis, Histoplasmosis, and Blastomycosis

R. F. HECTOR,[1] B. L. ZIMMER,[2] AND D. PAPPAGIANIS[2]*

Cutter Biological, Berkeley, California 94710,[1] and Department of Medical Microbiology, University of California Davis School of Medicine, Davis, California 95616[2]

Received 23 October 1989/Accepted 19 January 1990

Nikkomycins X and Z, competitive inhibitors of fungal chitin synthase, were evaluated as therapeutic agents in vitro and in mouse models of coccidioidomycosis, histoplasmosis, and blastomycosis. In vitro, the nikkomycins were found to be most effective against the highly chitinous, dimorphic fungi *Coccidioides immitis* and *Blastomyces dermatitidis*, were less effective against yeasts, and were virtually without effect on the filamentous fungus *Aspergillus fumigatus*. Additionally, by transmission electron microscopy, nikkomycin Z was highly disruptive to the cell wall and internal structure of the spherule-endospore phase of *C. immitis* in vitro. In vivo, nikkomycin Z was more effective than nikkomycin X, was also found to be superior on a milligram per milligram basis to the majority of azoles tested in the models of coccidioidomycosis and blastomycosis, and was moderately effective in histoplasmosis. A study of the pharmacokinetics in mice showed that nikkomycin Z was rapidly eliminated after intravenous infusion but that absorption after oral administration was sufficiently slow to allow inhibitory levels to persist for more than 2 h. Results of limited toxicology tests suggest that nikkomycin Z was well tolerated at the dosages employed.

Several antifungal compounds in use today affect the integrity of the fungal cytoplasmic membrane or the synthesis of its constituent ergosterol. Although some of these drugs have demonstrable efficacies and have been widely utilized for treatment of human patients and animals, there is a clear need for drugs with greater fungicidal activities. Because the cell walls of most medically important fungi contain chitin, a polymer not encountered in mammals, compounds which interfere with the synthesis or degradation of this polymer could be useful in therapy of mycoses (3). The polyoxins, which are specific inhibitors of fungal chitin synthase due to their similarity in structure to UDP-*N*-acetylglucosamine (reviewed in references 4 and 13), the substrate of chitin synthase, had previously been used in vitro against *Candida* spp. (1, 22, 31) and dimorphic fungi with some success (6, 17). A related group of compounds is the nikkomycins, which are structurally similar to the polyoxins and are also inhibitors of chitin synthase (3, 7). We employed nikkomycins X and Z (NX and NZ, respectively) in vitro against selected medically important fungi representing true yeast, dimorphic, and filamentous groups. Because of the high level of activity of these agents against the dimorphic pathogenic fungi, we subsequently evaluated them in mouse models of coccidioidomycosis, blastomycosis, and histoplasmosis, and the results are reported herein. In addition, data are provided on the pharmacokinetics and safety of these compounds.

MATERIALS AND METHODS

Drugs. NX and NZ and the triazole R 3783 were received as powders from Bayer AG, Wuppertal, Federal Republic of Germany; fluconazole was received as a powder from Pfizer Inc., Groton, Conn.; itraconazole was received as a powder from Janssen, Piscataway, N.J.; ketoconazole was purchased as Nizoral (200-mg tablets); and amphotericin B was purchased as Fungizone. For administration the nikkomycins and fluconazole were prepared as solutions in 0.1% agar (Difco Laboratories, Detroit, Mich.) with 0.5% glucose. R 3783 was prepared either as a suspension in 0.1% agar with 0.5% glucose or dissolved with heating at 60°C in polyethylene glycol 200 (J. T. Baker, Phillipsburg, N.J.). Ketoconazole tablets were pulverized and suspended in the glucose-agar. Itraconazole was dissolved in polyethylene glycol 200. The concentrations of the nikkomycins and azoles were adjusted for oral delivery in 0.1-ml volumes. Amphotericin B was diluted in 5% glucose and water and injected intraperitoneally in 0.1-ml volumes.

Organisms and conditions of culture. *Coccidioides immitis* Silveira (ATCC 28868) (University of California Davis collection), *Histoplasma capsulatum* G217B (obtained from G. Kobayashi, Washington School of Medicine, St. Louis, Mo.), *Blastomyces dermatitidis* 1389 (obtained from the University of Kentucky collection, Lexington), *Candida albicans* B311 (originally obtained from H. Hasenclever, National Institutes of Health, Bethesda, Md.), *Candida tropicalis* 1525 (obtained from J. Domer, Tulane School of Medicine, New Orleans, La.), *Cryptococcus neoformans* Blouin (University of California Davis collection), and *Aspergillus fumigatus* ATCC 13073 were used for all experiments. Arthroconidia of *C. immitis* were obtained by culturing the fungus on 2% glucose–1% yeast extract (GYE; Difco) agar at 35°C for several weeks, dislodging the arthoconidia into sterile water by using a magnetic stirring bar, and storing them in liquid suspension at 4°C until needed. The spherule-endospore phase was maintained in liquid culture as described by Levine et al. (21). Yeast-phase *B. dermatitidis* was maintained by weekly passage at 37°C on Kelley agar (20) modified by the substitution of glucose for starch and hemoglobin (equivalent to 0.5% [vol/vol] sheep blood) in place of serum. Inocula for experiments with the yeast phase were prepared by inoculation of fresh slants 72 h prior to experiments. *H. capsulatum* was maintained in the yeast phase on brain heart infusion-blood agar slants at 37°C by weekly transfer, with fresh slants inoculated 72 to 96 h before initiation of experiments. The *Candida albicans* and *Cryptococcus neoformans* isolates were stored at −70°C

* Corresponding author.

until needed, and inocula were propagated in GYE broth at 37°C overnight. Conidia of *A. fumigatus* were collected after growth on GYE agar at 37°C for 7 to 10 days and then were harvested and stored as a suspension in water at 4°C until needed.

Animals. Outbred CFW female mice (20 to 24 g, average weight; Charles River Breeding Laboratories, Inc., Portage, Mich.) were used in both survival and organ load experiments for coccidioidomycosis and blastomycosis; CF-1 or CFW mice were used for histoplasmosis.

Susceptibility testing. All tests were performed in broth by using microdilution plates. The yeasts were tested in yeast-nitrogen base (Difco) with glucose and asparagine, *B. dermatitidis* (yeast phase) and *A. fumigatus* were tested in Sabouraud glucose broth, and *C. immitis* (spherule-endospore phase) was tested in modified Converse medium as previously described (18). All cultures were incubated at 37°C. MICs were determined at 48 h for all but the dimorphic fungi, which were determined at 96 h.

Transmission electron microscopy. A spherule-endospore culture was initiated by seeding 250-ml Erlenmeyer flasks containing 100 ml of modified Converse medium to approximately 10^6 CFU of *C. immitis* endospores, which were incubated with shaking at 37°C. After 9 h of incubation, NZ was added to one flask to achieve a final concentration of 2 μg/ml, and the flasks were incubated an additional 24 h. The spherules were then preserved with Formalin (0.05% [vol/vol]) and processed for electron microscopy. The cells were embedded in Spurr medium, sectioned, and stained with osmium tetroxide and lead citrate. The sections were examined with a Philips EM 400 transmission electron microscope.

Experimental design. The therapeutic efficacy of the compounds was examined by their effects on the survival of infected mice and on the number of viable fungi in the target organs.

(i) Survival experiments. All survival experiments were designed to follow an acute course, with the first deaths generally occurring in 5 to 12 days. The purpose of this approach was to provide a rigorous test of the antifungal agent, with the outcome essentially determined before an active immune response could influence results. For the coccidioidomycosis pulmonary experiments, infection was by the respiratory route. Mice were first anesthetized with pentobarbital (50 mg/kg of body weight, intraperitoneally), and then 30 μl of inoculum (5×10^3 to 10×10^3 arthroconidia) was placed in the nares, from which it was inhaled. For the coccidioidal meningocerebral model, the inoculum of 60 to 90 conidia was delivered intracranially by puncturing the crania with a 27-gauge needle and delivering 30-μl samples of the fungal suspensions as shallowly as possible. For the blastomycosis experiments, the infection of the lungs was established by intravenous infection with 5×10^4 yeast-phase cells. For the histoplasmosis model, mice were infected intravenously with approximately 5×10^6 yeast-phase cells. Therapy was begun 48 h after inoculation. Nikkomycins were administered orally twice a day (b.i.d.) and azoles were given once a day (q.d.) for periods indicated in the figures. All animals were maintained for at least 28 days and monitored daily for mortalities. There were 10 mice per group.

(ii) Short-term organ load experiments. Mice were infected by the appropriate routes with sublethal to moderately lethal challenges (levels indicated in the figure legends). Groups of eight mice each were used in all experiments. Therapy was initiated 48 h later and was given b.i.d. for all nikkomycins and azoles and q.d. for amphotericin B. Therapy was given for 5 days, and after a 48-h washout period, all animals were sacrificed. For all experiments, a target organ(s) was removed and weighed and then homogenized in 10 ml of sterile phosphate-buffered saline, using a tissue homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.). Serial dilutions were made and plated in a quantitative fashion on the surface of Sabouraud glucose agar (Difco) containing 50 μg of chloramphenicol per ml and 50 μg of cycloheximide per ml (Sigma Chemical Co., St. Louis, Mo.) for *C. immitis* and *B. dermatitidis* and on brain heart infusion agar with chloramphenicol and cycloheximide for *H. capsulatum*. Cultures for *C. immitis* were incubated at 35°C, and for *B. dermatitidis* and *H. capsulatum* cultures were incubated at room temperature. Colonies were enumerated and data were calculated, with results expressed as the $\log_{10}$ CFU/g of tissue.

TABLE 1. MICs of nikkomycins against diverse fungi

Organism	MIC (μg/ml)[a]	
	NX	NZ
Coccidioides immitis	0.77	0.125
Blastomyces dermatitidis	8	30
Cryptococcus neoformans	125	250
Candida albicans	125–250	250–500
Candida tropicalis	>8,000	>8,000
Aspergillus fumigatus	>4,000	>4,000

[a] Read at 48 h except for dimorphic fungi, which were read at 96 h.

Bioassay. Groups of mice treated with NZ were bled into heparinized tubes at several intervals. After centrifugation, 200 μl of each plasma sample was precipitated with 400 μl of 95% ethanol as a means of inactivating peptidases. These samples (together with standards prepared by adding NZ to normal mouse plasma and treating it as described above) were again centrifuged to pellet the precipitate, and the supernatants were transferred to glass tubes (13 by 100 mm). The supernatants were evaporated by being heated in a water bath under a stream of N_2 and then reconstituted with 50 μl of distilled water.

Plates for the bioassay of NZ were composed of yeast-nitrogen base (Difco) with 1% glucose and 0.15% asparagine (Sigma) added to 1.5% molten agar. To increase the sensitivity of the assay by potentiating the action of NZ, the triazole R 3783, dissolved in a minimal volume of 95% ethanol, was added to the agar at a final concentration of 0.1 μg/ml. Just prior to pouring, log-phase *Candida albicans* B311 was added to the agar at a final concentration of 10^4 CFU/ml. Twenty-five milliliters of agar was added to each 150-mm-diameter petri plate and allowed to solidify. Wells of 6-mm diameter were punched with a cork borer, and 30-μl samples were added. The plates were incubated at 30°C, and the diameters of inhibitory zones were read after overnight incubation. Under these conditions, the range of linearity was 2 to 100 μg/ml.

Statistical analyses. Results from short-term organ loads were analyzed by Duncan's multiple range test, with significance determined at the $P = 0.05$ level. Survival data were analyzed by the Cox proportional hazards estimation, using one-sided tables in comparisons of control and treatment group values and two-sided tables in comparisons of the R 3783 groups with other treatment groups. Significance was determined at the $P = 0.05$ level. Data inappropriate for Cox analysis were compared in pairwise fashion, using the log-rank method with significance determined at the $P = 0.05$ level.

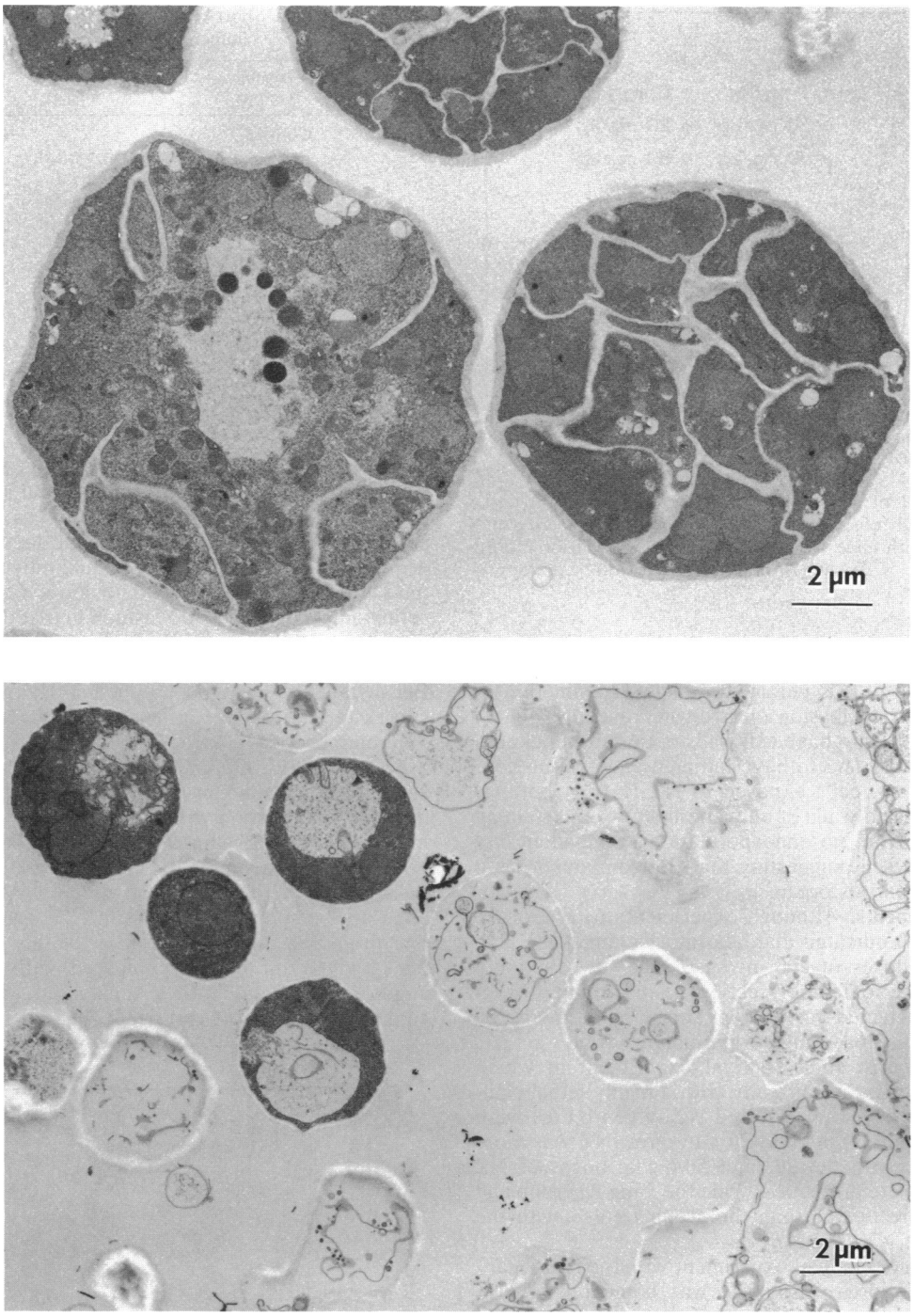

FIG. 1. Transmission electron micrograph of 33-h-old spherules of *C. immitis* cultured in vitro. (Top) Control, untreated spherules; (bottom) spherules of the same age but exposed to NZ at 2 μg/ml for 24 h. The treated spherules are much smaller and have thinner, electron-transparent walls, and most have marked damage to internal structures or are lysed.

RESULTS

Susceptibility testing. The results of MIC tests against six different species of fungi representing the dimorphic fungi (*C. immitis* and *B. dermatitidis*), true yeasts (*Candida albicans*, *Candida tropicalis*, and *Cryptococcus neoformans*), and a filamentous opportunistic pathogen (*A. fumigatus*) are presented in Table 1. As can be seen, NX and NZ showed the greatest inhibition against the dimorphic pathogenic fungi, with values in the microgram per milliliter range. Both agents had modest degrees of activity against some yeasts but were inactive against *A. fumigatus* in the milligram per milliliter range. The extreme resistance by *Candida tropicalis* was an unexpected but reproducible finding.

Electron microscopy. In order to assess the morphological effects of NZ on *C. immitis*, a synchronous spherule-endos-

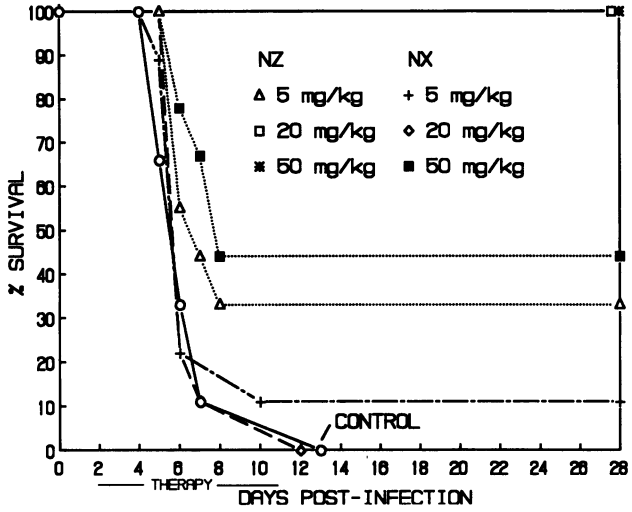

FIG. 2. Survival of mice with pulmonary coccidioidomycosis following intranasal infection with 9×10^3 CFU, comparing untreated controls with mice treated with NX and NZ at three dose levels given b.i.d. (5, 20, and 50 mg/kg). The period of therapy is indicated below the x axis.

pore culture was initiated, allowed to grow for 9 h, and then treated with 2 μg of NZ per ml for 24 h. Examination of control cells by transmission electron microscopy (Fig. 1, top) showed the cells to have cell walls of uniform thickness and electron density and to have initiated endosporulation. In marked contrast, cells exposed to NZ (Fig. 1, bottom) were smaller and had walls of uneven thickness and staining intensity. In addition, no endosporulation was evident and many cells had lysed, suggesting that the compound has a fungicidal effect on this organism.

In vivo experiments. Although experiments to assess the toxicology of NZ indicated that laboratory animals are able to tolerate oral doses of 400 mg/kg of body weight for a 30-day period (see toxicology section), a maximum dose of 75 mg/kg was selected for therapy because of favorable results seen in this dose range in preliminary trials.

Coccidioidomycosis. In a survival experiment in which animals were infected intranasally with a highly lethal challenge, three dose levels of NX and NZ (given b.i.d.) were administered, after a 48-h delay, for 10 days. NZ was more active than NX, with the 20- and 50-mg/kg doses of NZ resulting in complete protection while the 5-mg/kg group had only slightly more deaths than the group treated with the 50-mg/kg level of NX (Fig. 2).

In a short-term organ load experiment with the pulmonary model of coccidioidomycosis, NZ was compared with R 3783 and fluconazole. The results from that experiment (Table 2) indicate that NZ at 50 mg/kg of body weight was able to eradicate nearly all of the fungus from the lungs of treated animals (only a single colony was recovered from a total of eight mice treated) and was therefore comparable to 25 mg of the triazole R 3783 per kg and superior to fluconazole under the conditions employed. In a second experiment in the same model, 20 mg of NZ per kg given b.i.d. was compared with 50 mg of the same drug per kg given q.d. The data indicate that divided doses are more effective than one dose per day, which is likely a reflection of the short half-life of this drug (see section on pharmacokinetics).

A model of meningocerebral coccidioidomycosis in mice was used to assess the ability of NZ to penetrate into the central nervous system and combat the fungus in what is considered the most deadly coccidioidal syndrome. A dosage of 50 mg of NZ per kg given b.i.d was compared with 25 mg of R 3783 per kg given q.d. for 21 days. While the onset of mortalities was earlier in the nikkomycin group (Fig. 3) than in the azole group, the data suggest that the azole was acting only in a fungistatic fashion in that deaths occurred in the latter group soon after cessation of therapy, whereas the numbers of deaths in the NZ group were ultimately fewer. Because of the early deaths in the NZ group, the survival curves for the two treatment groups were not statistically different.

TABLE 2. Short-term organ loads with lungs of mice infected with *C. immitis*[a]

Expt	Treatment group	Dose (mg/kg)	Mean log CFU/g ± SEM
1	Control		6.35 ± 0.06
	R 3783	2.5 b.i.d.	4.07 ± 0.92
	R 3783	10 b.i.d.	2.88 ± 0.45
	R 3783	25 b.i.d.	0.00
	Fluconazole	2.5 b.i.d.	3.77 ± 1.12
	R 3783	10 b.i.d.	4.42 ± 0.66
	R 3783	25 b.i.d.	2.62 ± 0.82
	NZ	50 b.i.d.	0.37 ± 0.37
2	Control		6.21 ± 0.15
	NZ	20 b.i.d.	1.12 ± 0.56
	NZ	50 q.d.	3.63 ± 0.62

[a] Animals were infected intranasally with 4,000 CFU.

Blastomycosis. A model of systemic blastomycosis with acute nodular pulmonary disease was established in mice by intravenous infection with an inoculum which caused 100% mortality in control animals in less than 10 days. Again, both NX and NZ were employed at three dose levels to compare their relative activities. In this model both compounds had good dose-response effects, with NZ again the more effective drug (Fig. 4). In a variation of this model, NZ at 20 mg/kg given b.i.d. was compared with four azoles and amphotericin B given at 1 mg/kg q.d., with therapy not administered until the first deaths had occurred on day 6. In

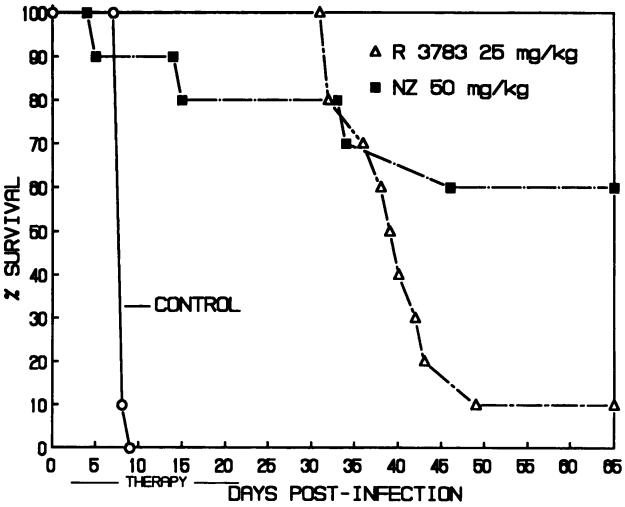

FIG. 3. Survival of mice with meningocerebral coccidioidomycosis infected intracranially with 90 CFU, comparing controls with mice treated with 50 mg of NZ per kg b.i.d. and 25 mg of R 3783 per kg q.d. The period of therapy is indicated below the x axis.

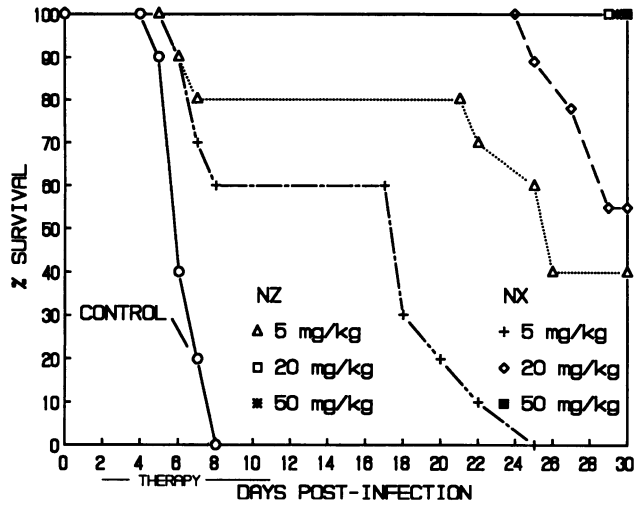

FIG. 4. Survival of mice with systemic blastomycosis infected intravenously with 4.9×10^4 CFU, comparing untreated controls with mice treated with NX and NZ at three dose levels given b.i.d. (5, 20, and 50 mg/kg). The period of therapy is indicated below the x axis.

this severe test of the ability of a drug to overcome advanced disease, NZ-treated mice had the fewest deaths, with no deaths occurring after day 8, i.e., 2 days after commencement of therapy, but the survival curve was not statistically superior to those from the R 3783 and amphotericin B groups (Fig. 5). However, survival in the NZ group was superior to that in the other azole-treated groups.

In a short-term organ load experiment, three dose levels of NZ were compared with four azoles and amphotericin B (Table 3). The high degree of activity of this agent against blastomycosis is evident, as the lowest dose of NZ tested (5 mg/kg) was as active as amphotericin B at 1 mg/kg and the most active azole (R 3783) at 25 mg/kg. The 50-mg/kg dose of NZ resulted in the sterilization of the lungs of most of the animals.

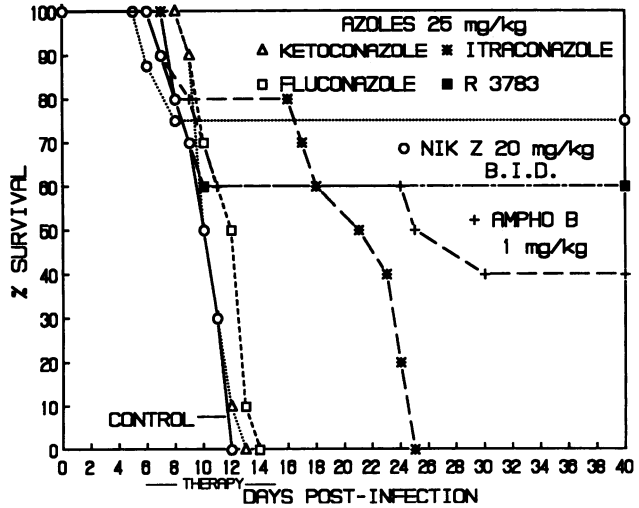

FIG. 5. Delayed-therapy study in systemic blastomycosis model in mice infected intravenously with 4.9×10^4 CFU, comparing controls with mice treated with ketoconazole, fluconazole, itraconazole, and R 3783 at 25 mg/kg q.d. and 1 mg of amphotericin B (AMPHO B) per kg given q.d. with NZ (NIK Z) at 20 mg/kg b.i.d. The period of therapy is indicated below the x axis.

TABLE 3. Short-term organ loads in lungs of mice infected with *B. dermatitidis*[a]

Treatment group	Dose (mg/kg)	Mean log CFU/g ± SEM	Stat group[b]
Control		6.19 ± 0.04	A
Ketoconazole	25	6.08 ± 0.12	A
Fluconazole	25	5.63 ± 0.12	A
Itraconazole	25	4.03 ± 0.23	B
Amphotericin B	1[c]	3.09 ± 0.23	C
R 3783	25	2.29 ± 0.37	D
NZ	5	2.43 ± 0.37	D
NZ	20	1.81 ± 0.57	D
NZ	50	0.37 ± 0.37	E

[a] Animals were infected with 100,000 CFU intravenously.
[b] Groups with different letters are statistically different at the $P = 0.05$ level.
[c] Amphotericin B given q.d. only; other drugs b.i.d.

Histoplasmosis. Three dose levels of NZ were used in a survival model in mice infected intravenously with *H. capsulatum*. The results show NZ at the low dose levels of 5 and 20 mg/kg of body weight to be highly effective in preventing deaths, as all animals survived (Fig. 6). The results from an experiment for short-term organ loads are presented in Table 4 and show that although a dose-response effect was seen, the moderate amount of clearance does not compare with that seen with the models of coccidioidomycosis and blastomycosis. However, the reduction in CFU per gram was similar to that seen in the fluconazole-treated animals.

Pharmacokinetics. A bioassay for NZ was developed and used for pharmacologic studies in mice. Animals were treated either orally or intravenously with 100 mg of NZ dissolved in phosphate-buffered saline per kg of body weight. Groups of three animals each were bled at intervals over a 2-h period and processed as described in Materials and Methods. The results of intravenous administration indicated that after the initial concentration in serum of 320 μg/ml the compound was rapidly eliminated, with a half-life of approximately 10 to 15 min. Results from oral administration with 100 mg/kg, however, suggest that the compound is

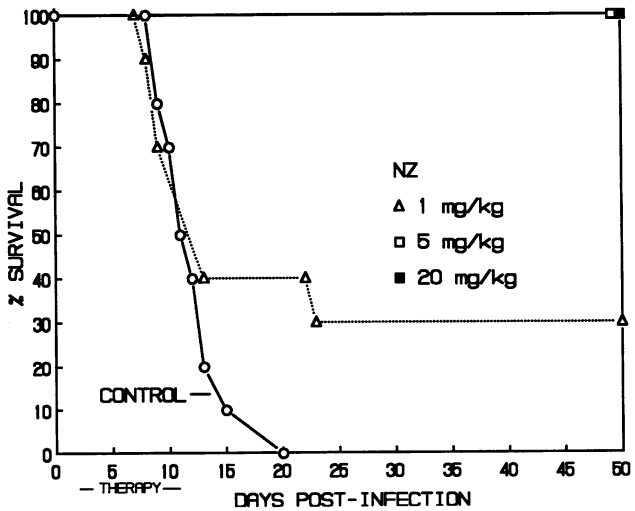

FIG. 6. Survival of mice with systemic histoplasmosis infected intravenously with 10^7 CFU, comparing untreated controls with mice treated with NZ at three dose levels given b.i.d. (5, 20, and 50 mg/kg). The period of therapy is indicated below the x axis.

TABLE 4. Short-term organ loads in livers and spleens of mice infected with *H. capsulatum*[a]

Treatment group	Dose (mg/kg)	Mean log CFU/g ± SEM		Stat group(s)[b]	
		Liver	Spleen	Liver	Spleen
Control		6.20 ± 0.13	5.54 ± 0.07	A	A
NZ	20	5.25 ± 0.10	5.16 ± 0.16	B	A, B
Fluconazole	25	5.34 ± 0.12	4.71 ± 0.10	B	B, C
NZ	50	4.37 ± 0.38	4.46 ± 0.14	C	C
R 3783	25	2.46 ± 0.12	0.96 ± 0.47	D	D

[a] Animals were infected with 850,000 CFU.
[b] Groups with the same letter are not statistically different at the $P = 0.05$ level.

slowly absorbed, with a peak concentration of 10 μg/ml occurring approximately 45 min after administration. Though the number of datum points are limited, the half-life appears to be nearly 1 h under these conditions.

Toxicology. A limited safety trial was conducted in mice by using agar vehicle or NZ given orally q.d. at 100 or 400 mg/kg for a 28-day period in groups of 15 each. Weights were recorded twice weekly and showed no discernible differences over the test period among the three groups. At the end of the test period, 10 animals from each group were bled and necropsied. Routine blood counts and chemistry panels (the latter done on pools of three mice each for sufficient volume) revealed no differences among treated groups and untreated controls. Samples for pathology were sent to an independent pathology service for assessment, with the only unusual finding being a comparatively increased incidence of gliosis in the brains of mice receiving the 400-mg/kg dose. Animals not sacrificed were held an additional 60 days for observation, with no outward differences apparent among the three groups.

DISCUSSION

The potential for using chitin as a chemotherapeutic target in fungi has been recognized for a number of years (3, 7, 14), though the reports in the literature are essentially limited to in vitro testing. Previously, reports have shown polyoxins, compounds structurally related to the nikkomycins, to be highly active against dimorphic, highly chitinous fungi (6, 17) and to have some activity against *Candida albicans* (1, 22, 31). In the present study we have demonstrated that the nikkomycins, in particular NZ, have pronounced activity against selected dimorphic fungal pathogens in animal models of these mycoses. Indeed, with the models of pulmonary coccidioidomycosis and systemic blastomycosis, NZ appears to be fungicidal. In particular, the experiment with delayed therapy in the blastomycosis model demonstrated the potency of this agent in reversing advanced disease. NZ was less effective in the model of coccidioidal meningocerebral disease; however, this may be a result of inadequate penetration into the central nervous system because of the limited half-life of this substance. With either continuous infusion or direct injection of the drug into the cerebrospinal fluid compartment, the outcome in treatment of this syndrome may be more favorable.

Unlike the dimorphic fungal pathogens mentioned above, in which chitin represents 10 to 20% of their cell walls in the parasitic phase (9, 11, 12, 19, 27, 29, 30), *Candida albicans* has a much smaller chitin component in its cell wall (5). Although small amounts of chitin can be found in the inner layers of the yeast cell wall (29), the highest concentration of the polymer is found in the yeast bud septum (2). This may partially explain the previous findings of Becker et al. (J. M. Becker, S. Marcus, J. Tallock, D. Miller, E. Krainer, R. K. Khare, and F. Naider, Letter, J. Infect. Dis. **157**:212–214, 1988), in which NZ was unable to prevent deaths in mice infected with a lethal challenge of *Candida albicans*. An additional factor which must be considered is the differences among different fungi in permeability and/or transport of these drugs intracellularly (22, 31).

With the MICs and the results of the animal experiments against the dimorphic fungi considered simultaneously, this report and the previous findings for cilofungin, the inhibitor of beta-glucan synthesis (J. R. Graybill, J. Ahrens, and M. Rinaldi, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1527, 1988), provide evidence that in vitro susceptibility tests for compounds active against the cell walls of fungi may be of predictive value for therapeutic outcome in mycoses. With azole antifungal agents, which are directed against the fungal cytoplasmic membrane, a correlation of susceptibility tests with experimental or clinical outcome has not been established (24).

The findings reported herein also give important clues as to the varying importance of chitin in the cell walls of different fungi. While much of the evidence is circumstantial, these and previous findings (17) suggest that the amount of chitin in the cell wall is not the sole determinant of susceptibility. Indeed, the filamentous fungi often have as much of this polymer in the cell wall as the susceptible dimorphic pathogens (25, 26), but the results of the MIC determination showed *A. fumigatus* to be refractory to the nikkomycins. Previously, we had shown that the spherule-endospore phase of *C. immitis* was susceptible to a closely related chitin synthase inhibitor, polyoxin D, whereas the filamentous phase was apparently resistant (17). Thus, in certain fungi, other wall polymers are likely able to substitute for any deficiencies created by inhibitors of cell wall synthesis. This was demonstrated to be the case with *Paracoccidioides brasiliensis* in the mycelial phase, which synthesized additional alpha-glucan in response to treatment with papulacandin B, an inhibitor of beta-glucan synthesis (8). With the parasitic phases of *C. immitis* and *B. dermatitidis*, however, the need for chitin may be more critical. As has been previously shown, chitin is the first polymer synthesized in the cell wall during progressive cleavage of *C. immitis* spherules, part of the process involved in replication, with treatment by polyoxin D halting this process completely (16, 17). In the present study, NZ was found to have the same effect, with inhibition of progressive cleavage and lysis of cells evident. Interestingly, the MIC of the nikkomycins for *C. immitis* is approximately equal to the calculated K_i value for this agent against the chitin synthase enzyme isolated from diverse fungi (3, 10, 15, 22, 23).

In conclusion, we have demonstrated that NX and NZ are efficacious against mycoses caused by the highly chitinous, dimorphic fungal pathogens, exceeding the effectiveness of certain azoles and amphotericin B under the conditions employed. In the ranges tested, the nikkomycins appear to have no adverse effects, as assessed by a limited study, and thus may compare favorably with antifungal agents currently in use. Additionally, the findings confirm that the cell wall is a suitable and specific target for antifungal drugs, inviting continued efforts for development of effective and safe therapy of the mycoses.

ACKNOWLEDGMENT

We thank Robert Munn for expert performance of electron microscopy.

LITERATURE CITED

1. **Becker, J. M., N. L. Covert, P. Shenbagmurthi, A. S. Steinfeld, and F. Naider.** 1979. Polyoxin D inhibits the growth of zoopathogenic fungi. Antimicrob. Agents Chemother. **23**:926–929.
2. **Braun, P. C., and R. A. Calderone.** 1978. Chitin synthesis in *Candida albicans*: comparison of yeast and hyphal forms. J. Bacteriol. **133**:1472–1477.
3. **Brillinger, G. U.** 1979. Metabolic products of microorganisms. 181. Chitin synthase from fungi, a test model for substances with insecticidal properties. Arch. Microbiol. **121**:71–74.
4. **Cabib, E.** 1988. The synthesis and degradation of chitin. Adv. Enzymol. Relat. Areas Mol. Biol. **59**:59–101.
5. **Chattaway, F. W., and M. R. Holmes.** 1968. Cell wall composition of the mycelial and blastospore forms of *Candida albicans*. J. Gen. Microbiol. **51**:367–376.
6. **Cooper, C. R., J. L. Harris, C. W. Jacobs, and P. J. Szaniszlo.** 1984. Effects of polyoxin AL on cellular development in *Wangiella dermatitidis*. Exp. Mycol. **8**:349–363.
7. **Dahn, U., H. Hagenmaier, H. Hohne, W. A. Konig, G. Wolf, and H. Zahner.** 1976. Nikkomycin, ein neuer Hemmstoff der Chitinsynthese bei Pilzen, Arch. Mikrobiol. **107**:143–160.
8. **Davila, T., G. San-Blas, and F. San-Blas.** 1986. Effect of papulacandin B on glucan synthesis in *Paracoccidioides brasiliensis*. J. Med. Vet. Mycol. **24**:193–202.
9. **Davis, T. E., Jr., J. E. Domer, and Y. T. Li.** 1977. Cell wall studies of *Histoplasma capsulatum* and *Blastomyces dermatitidis* using homologous and heterologous enzymes. Infect. Immun. **15**:978–987.
10. **Delzer, J., H. P. Fiedler, H. Muller, H. Zahner, R. Rathmann, K. Ernst, and W. A. Konig.** 1984. New nikkomycins by mutasynthesis and directed fermentation. J. Antibiot. **37**:80–82.
11. **Domer, J. E.** 1971. Monosaccharide and chitin content of cell walls of *Histoplasma capsulatum* and *Blastomyces dermatitidis*. J. Bacteriol. **107**:870–877.
12. **Domer, J. E., J. G. Hamilton, and J. C. Harkin.** 1967. Comparative study of the cell walls of the yeastlike and mycelial phases of *Histoplasma capsulatum*. J. Bacteriol. **94**:466–474.
13. **Farkas, V.** 1979. Biosynthesis of cell walls of fungi. Microbiol. Rev. **43**:117–144.
14. **Gooday, B. W.** 1977. Biosynthesis of the fungal wall—mechanisms and implications. The first Fleming Lecture. J. Gen. Microbiol. **99**:1–11.
15. **Gow, L. A., and C. P. Selitrennikoff.** 1984. Chitin synthetase of *Neurospora crassa*: inhibition by nikkomycin, polyoxin B, and UDP. Curr. Microbiol. **11**:211–216.
16. **Hector, R. F., and D. Pappagianis.** 1982. Enzymatic degradation of the walls of spherules of *Coccidioides immitis*. Exp. Mycol. **6**:136–152.
17. **Hector, R. F., and D. Pappagianis.** 1983. Inhibition of chitin synthesis in the cell wall of *Coccidioides immitis* by polyoxin D. J. Bacteriol. **154**:488–498.
18. **Hector, R. F., B. L. Zimmer, and D. Pappagianis.** 1988. Microtiter method for MIC testing with spherule-endospore-phase *Coccidioides immitis*. J. Clin. Microbiol. **26**:2667–2668.
19. **Kanetsuna, F., L. M. Carbonell, F. Gil, and I. Azuma.** 1974. Chemical and ultrastructural studies on the cell walls of *Histoplamsa capsulatum*. Mycopathol. Mycol. Appl. **54**:1–13.
20. **Kelley, W. H.** 1939. A study of the cell and colony variations of *Blastomyces dermatitidis*. J. Infect. Dis. **64**:292–296.
21. **Levine, H. B., J. M. Cobb, and C. E. Smith.** 1960. Immunity to coccidioidomycosis induced in mice by purified spherule, arthrospore, and mycelial vaccines. Trans. N. Y. Acad. Sci. **20**:436–449.
22. **McCarthy, P. J., P. F. Troke, and K. Gull.** 1985. Mechanism of action of nikkomycin and the peptide transport system of *Candida albicans*. J. Gen. Microbiol. **131**:775–780.
23. **Mueller, H., R. Furter, H. Zahner, and D. Rast.** 1981. Metabolic products of microorganisms. 203. Inhibition of chitosomal chitin synthetase and growth of *Mucor rouxii* by nikkomycin Z, nikkomycin X, and polyoxin A: a comparison. Arch. Microbiol. **130**:195–197.
24. **Odds, F. C.** 1988. Candida and candidosis, p. 280–282. Bailliere Tindall, London.
25. **Pollack, J. H., C. F. Lange, and T. Hashimoto.** 1983. "Nonfibrillar" chitin associated with walls and septa of *Trichophyton mentagrophytes* arthrospores. J. Bacteriol. **154**:965–975.
26. **Porter, C. A., and E. Jaworski.** 1966. The synthesis of chitin by particulate preparations of *Allomyces macrogynus*. Biochemistry **5**:1149–1154.
27. **Reiss, E.** 1977. Serial enzymatic hydrolysis of cell walls of two serotypes of yeast-form *Histoplasma capsulatum* with $\alpha(1\rightarrow3)$-glucanase, $\beta(1\rightarrow3)$-glucanase, pronase, and chitinase. Infect. Immun. **16**:181–188.
28. **Tronchin, G., D. Poulain, J. Herbaut, and J. Biguet.** 1981. Localization of chitin in the cell wall of *Candida albicans* by means of wheat germ agglutinin. Fluorescence and ultrastructural studies. Eur. J. Cell. Biol. **26**:121–128.
29. **Wheat, R., T. Terai, A. Kiyomoto, N. F. Conant, E. P. Lowe, and J. Converse.** 1967. Studies on the composition and structure of *Coccidioides immitis* cell wall, p. 237–242. *In* L. Ajello (ed.), Coccidioidomycosis. University of Arizona Press, Tucson.
30. **Wheat, R. W., C. Tritschler, N. F. Conant, and E. P. Lowe.** 1977. Comparison of *Coccidioides immitis* arthrospore, mycelium, and spherule cell walls, and influence of growth medium on mycelial cell wall composition. Infect. Immun. **17**:91–97.
31. **Yadan, J.-C., M. Gonneau, P. Sarthou, and F. Le Goffic.** 1984. Sensitivity of nikkomycin Z in *Candida albicans*: role of peptide permeases. J. Bacteriol. **160**:884–888.